

nature

17 January 1980

A new responsibility for science

A major impact of public reaction to the Soviet invasion of Afghanistan has been to legitimate the slowing down — and in some cases even the reversal — of efforts to limit the global spread of nuclear weapons. The most obvious manifestation of this process has been President Carter's decision to defer consideration by the US Senate of the new Strategic Arms Limitation Treaty (SALT II), concluded last year after lengthy negotiations with the USSR. But the impact of the Soviet moves is already making itself felt across a wide range of non-proliferation initiatives — and the net result can only be to bring the threat of nuclear war a step nearer.

It seems inevitable, for example, that the new strains on East-West relations will provide one more hurdle to the successful negotiation of a comprehensive test ban, severely decreasing hopes for an early agreement. In Pakistan, US moves to cut off military and economic aid last year in an attempt to wean the country away from nuclear weapons production are now being reversed to help provide a counterweight to the Soviet presence in nearby Afghanistan. And to mollify Pakistan's own neighbour, India, State Department officials in Washington are said to be dropping their opposition to the export of highly-enriched nuclear fuels for the Tarapur nuclear plant, despite India's continued refusal to accept international safeguards.

To warn of the dangers inherent in these moves is in no way to condone the Soviet Union's actions, nor to deny that the USSR must share a large part of the blame for their implications. But as far as US policy is concerned, it is important that the realities of the situation are not confused with images of that reality.

The paradox of President Carter's position, for example, is

that by taking a firm stand on international issues such as Iran and Afghanistan, he has regained a reputation as a political leader previously lost for failures in relatively unrelated domestic spheres, namely energy and rising inflation. Furthermore the defense industry had not been slow to point out the domestic merits of increased military spending. Pentagon officials, for example, point out that in the past defense-sponsored research has provided a major boost to civilian technologies — and that given current national concerns about declining rates of innovation, increasing the military R&D budget could again help efforts in this direction.

The volatility of US politics tends to create a rhetoric designed primarily for domestic consumption. And in many ways the potential political gains that might have been achieved by supporting SALT have been overtaken by the gains of undercutting the same agreement.

No-one can pretend that the way forward for arms control is clear. But one strategy that could be pursued with vigour would be to separate discussions aimed at limiting nuclear weapons as far as possible from the temporarily sinking ship of *détente*. In the past, the scientific community has ably taken on this role, using its position on the fringes of the political establishment to keep open lines of communication that might otherwise be lost. Last week the council of the American Association for the Advancement of Science passed a resolution emphasising the continued need to support arms control efforts, and drawing attention to the particular responsibility of scientists to help this process (see page 234). We support the AAAS's efforts, and urge British scientists to do likewise. □

No engineers without teachers

At last the Finniston report on the training and status of engineers in Britain has been officially published. The history of leaks of the report has been so long that it is rather an anticlimax to have the real thing on one's desk*; *Nature* described the bulk of its recommendations last summer (2 August 1979, page 352). We support most of them as indicating a practical, as opposed to big-headedly political route towards the improvement of Britain's economic competitiveness. Britain has too long neglected and rejected the applied scientist and engineer as a creator of wealth; the paradigm of economic activity has been the City, where paper rather than materials are moved: a second-order economic activity. There is a strong element of class-consciousness in this anti-technical valuation, and it pervades the whole of the establishment, including the Civil Service. Even the Department of Industry is neglectful of technological change.

Perhaps Finniston can blow a wind of change through all this — but it is unlikely. A sense of depression is increased if one looks at the figures for the numbers of science teachers in training in England and Wales — for these are the people who will influence the outlook of the next generation. Take physics and chemistry, for example. Of 19,500 physics teachers in secondary schools in January 1977, only 43% were actually qualified to teach physics. Of 18,800 chemistry teachers, half were qualified. The majority of teachers were 'filling-in', when their basic training was in another subject. In January 1979, the annual number of new physics teachers needed to fill vacancies and begin to replace unqualified teachers was estimated by the Department of Education and Science to be 1,300. In chemistry the number was 600.

Against this, in 1979 just 244 physics and 345 chemistry graduates entered one-year DipEd courses to learn to teach —

satisfying roughly one fifth of the need for physicists and one half that for chemists.

Mathematics is worse and biology better off than this. Of 48,300 maths teachers (maths is a compulsory subject), 62% are qualified, and there is an annual need (DES figures) of 4,300. The input of graduate trainees in 1979 was 636 — one seventh the need. In biology there are 20,900 teachers of whom 55% are qualified; the annual need is 600; and the 1979 graduate input was 657, satisfying demand.

In all subjects the position is worsening. Three years previously there were 412 graduate physics entrants into teacher training (244 in 1979); 438 chemists (345 in 1979) and 800 mathematicians (636 in 1979). (Biology fell slightly from 691 in 1976 to 657 in 1979.)

Without well-qualified science teachers, there is little hope for the future of science and engineering in Britain. Now is also time to discuss how to attract good science teachers. □

*'Engineering our future'. Report of the Committee of Inquiry into the engineering profession. HMSO, £5.

Editor of Nature

NATURE is pleased to announce the appointment of Mr John Maddox as Editor. Mr Maddox is presently Director of the Nuffield Foundation and was Editor of *Nature* from 1966 to 1973. He will take up his appointment not later than 30 June. MacMillan Journals are particularly appreciative of *Nature's* development during Dr Davies' editorship and it is Mr Maddox's intention to build on his achievements.



United States

David Dickson reports from the annual conference of the American Association for the Advancement of Science in San Francisco

Council supports arms control

FOLLOWING a public meeting at which a number of scientists described the increasingly urgent need to limit the proliferation of nuclear weapons, the council of the American Association for the Advancement of Science agreed last week to establish a working group "to help organise and mobilise resources towards nuclear arms control."

The AAAS council also agreed that efforts for directing science towards peace rather than war should be a major theme of next year's annual meeting, which will take place in Toronto.

Both decisions formed part of a resolution on nuclear weapons control submitted by seventeen members of the association, which pointed out that "improving the effectiveness of science in the promotion of human welfare is an objective and a special responsibility of the

AAAS."

By adopting the resolution, the AAAS council agreed to support both US efforts to obtain effective bilateral nuclear arms limitations and the completion of the

Gene resource programme called for

In a further resolution, the AAAS council gave its support to efforts to develop a national gene resource conservation programme. A resolution was passed which argued that there is insufficient support in the US for addressing the rapid depletion of germplasm resources of natural ecosystems, and that there is currently no comprehensive or effective national programme which would ensure that important germplasm resources are safeguarded.

Comprehensive Test Ban Treaty. It also expressed opposition to "the development by any country of new weapons systems which make verification more difficult, or pose a first-strike threat."

Supporters of the resolution point out that the latter is an implicit reference to, among other things, the recent decision by President Carter to recommend the deployment of a new generation of Minuteman missile, the MX.

The council also agreed to support the development of plans for the step-by-step conversion by all nuclear-weapons-producing nations of facilities for nuclear weapons production, research and testing, into science and technology facilities for peaceful uses. Such "conversion" plans are expected to be made the subject of a special session at next year's meeting of the association. □

Military support of basic research defended

RECENT increases in military support for basic research are rekindling the fierce debate over its social and ethical implications dormant for much of the past decade following widespread criticism during the Vietnam war era.

Supporters of military funding for science, addressing a session of the AAAS meeting, not only emphasised its importance for the long-term development of new weapons systems, but also claimed that through technological spin-offs — which have ranged from synthetic rubber to large-scale integrated circuits — military-supported research can help stimulate general industrial innovation in the US.

Critics, however, argued that the military control of research meant such research would inevitably be skewed towards the production of weapons of mass destruction, and was therefore in fundamental conflict with the well-being of mankind.

Dr George Gamota, director for research in the office of the Under-Secretary of Defense for Research and Engineering, told the audience that the department was trying hard to repair the working relationship with the scientific and engineering communities that had been weakened in the mid-1960s and early 1970s.

Particular emphasis was being paid to restoring defense support for university research. Forty per cent of the basic research currently funded by DoD was carried out by universities; and over the past three years, university support

increased by nearly 70%, although overall support for basic research increased by only 30%.

The need for military support for basic science, said Dr Gamota, "is rendered even more urgent by the relative decline in the extent of basic research conducted internally by industry or supported by the industrial sector on the academic community."

Referring to the Mansfield Amendment, passed in 1971 and restricting DoD support to research with a potential relationship to military needs, Dr Gamota said there was nothing in the act which touched upon the department's ability to support basic research. "Only the availability of funding and the level of interest of other agencies determines how wide a spectrum of research support is possible in the context of the DoD mission," he said.

Dr Gamota argued that it was impossible to single out any major basic research effort, particularly a new field, that could be categorically ruled out as potentially irrelevant to the Defense Department. "Our job is to follow the national trend and ensure that adequate funding is provided in areas of potential interest to DoD, and primarily only funding limitations and the extent of other agency interests limit our scope". The aim and goal of the department's support of basic research was "to ensure that the support will keep us technologically ahead of the world."

A full-blooded attack on such thinking, however, came from Professor George

Wald, emeritus professor of biology at Harvard University, who accused military planners of helping create a "mad nightmare of a world in which we are putting our children."

In contrast to those who argued that the US was now lagging behind the USSR in military technology, Professor Wald said that America was still five years ahead, both qualitatively and quantitatively. And although he admitted that he had no confidence in the peaceful intentions of the Soviet Union, it was the US, he said, which was currently leading the arms race.

Earlier in the conference, Professor Philip Morrison of the Massachusetts Institute of Technology, argued that through a mixture of judiciously chosen policies it should be possible to cut the military budget by 40% to 50% while preserving the same level of national security as exists at present.

"The key point is to buy what we need, and organise to use it as we need. We need not buy systems because one or another service or firm wants us to do so, or because once in another world they were useful, or because the Soviet or someone else has them," Professor Morrison said.

Recent events at the global and domestic level made it important that scientists in the AAAS and elsewhere take a close look at science in the service of war. "The US has largely paced the arms race," he said. "We can cut military research and development to a reasonable size, and restore the talent and resources thus set free to the growing needs of civil society." □

Nuclear accident casts a long shadow

EVIDENCE that last March's accident at the Three Mile Island nuclear power plant has already cast a long shadow over public attitudes towards a broad range of activities associated with nuclear power was presented by various speakers to last week's meeting of the American Association for the Advancement of Science.

Speaking at a session on risk in technological society, Dr Peterson, until recently director of the US Congress' Office of Technology Assessment and a member of the public commission established by President Carter to report on the accident, said that in the climate following the accident, public reaction to any incident involving a major release of radioactivity into the environment would almost certainly lead to demands for an end to the use of nuclear energy. "If in the interim we have tied our way of life to electricity from nuclear, the economic and social costs will be major indeed," he warned.

Dr Peterson drew attention to recent recommendations that plans for evacuating the residents close to a nuclear plant be developed before a plant is authorised to operate. Several nuclear physicists with the Nuclear Regulatory Commission, for example, had recently said that in the event of a future nuclear plant accident — and given the same amount of information as was available at the TMI incident — they would again recommend evacuation.

"Together these facts demonstrate that when individuals have the opportunity to dig deeply into the operation of nuclear

power plants, they conclude that it is only a matter of time before an accident releasing a devastating amount of radioactive material occurs. Why else would we need evacuation plans?" he asked.

Dr Peterson suggested that although the potential for a catastrophic accident raised the most amount of public concern, a more serious risk in the long run could come from the cumulative impact of countless minor releases of radioactivity. These ranged from uranium mill tailings, through the disposal of radioactive wastes, to the use of part-time employees as "sponges" to carry out brief tasks in highly contaminated areas to save regular employees from excessive exposure.

Another lesson discovered recently, he said, was that the nuclear industry had not learnt its lesson. He quoted, for example, advertisements that had been placed in major newspapers by the Edison Electric Institute, claiming that the electric companies agreed with the Kemeny Commission's message that nuclear power could proceed, but with caution.

"The Kemeny Commission said no such thing," protested Dr Peterson. "The introduction to our report carefully explains that our assignment limited us to matters bearing directly on the Three Mile Island accident, and that we made no judgements on a number of other issues — for example, the disposal of radioactive wastes — that must be considered in deciding whether nuclear power is basically safe."

A different slant on the impact of increased public concern about nuclear hazards was given by Nobel prizewinner Dr

Rosalyn Yalow in the course of a public lecture on radioimmunoassay.

Dr Yalow referred in particular to the recent press coverage that accompanied the decision by various states temporarily to close radioactive waste disposal sites. She suggested that the so-called problem of radioactive waste disposal from hospitals and medical centres was really not a problem but rather a figment of the "small minds and fertile imaginations" of various regulatory agencies.

"An unreasonable fear of any level of radiation associated with radioactivity or nuclear energy generated by newspaper headlines and publicity seekers was now pervasive throughout our society," Dr Yalow said. "Because of this we dissipate research talent and funding on make-work projects in response to unreasonable fears in make-work situations."

She cited, for example, the recent announcement that a number of research groups are to study possible long-range health effects secondary to the reactor accident at Three Mile Island. The maximum estimated dose received by anyone living near the reactor during the accident was about 70 millirems, less than one third the amount they would have received by spending a year in Colorado.

"If these were the maximal doses received one can predict with absolute certainty that, other than psychological effects, no physical aberration would be demonstrable. Yet we waste time, money and research talent — and generate more psychological stress than would otherwise occur in unnecessary make-work of no scientific merit." □

Women scientists still lose out to men

DESPITE almost a decade of affirmative action and raised consciousness, women continue to face a number of barriers in pursuing a scientific career, according to Mrs Betty Vetter, executive director of the Scientific Manpower Commission.

Speaking at a session on employment opportunities for science and engineering doctorates, Mrs Vetter said that women had approximately doubled their share of doctorates in science and engineering since 1970. But they still tended to be employed in lower-paid jobs than men, to have more difficulty in gaining tenure at universities, and to suffer a much higher rate of unemployment.

"It is not at all uncommon to find persons who believe that affirmative action programmes for women or for minorities have given women an edge in employment opportunities, but the statistics do not seem to bear out an assumption of equal treatment, even at the entry level," she said.

For example, while it might be expected

that women who obtained their PhDs in the 1950s and 1960s had not advanced as rapidly as men from the same cohorts, one might expect to find that those from more recent classes would be advancing at rates comparable to men: yet evidence produced by a survey carried out last year by the National Academy of Sciences showed that this was not true.

In the life sciences, for example, 23% of academically-employed men earning PhDs in the period 1970-74 had been appointed assistant professor by 1977, but only 11% of women in academic careers earning PhDs in the same period. In contrast, 27.5% of the women PhDs held the rank of lecturer or instructor, compared to 15.5% of the men.

In the federal government, a number of studies had shown that women still tend to advance in their careers more slowly than men, overall lagging by an average of 1½ to 2 civil service grades. For example, in 1978 women made up almost 31% of all microbiologists employed by the federal

government, but their median grade — and their salary average — was about 20% less than that of their male counterparts.

The best possibility for reducing inequity of opportunity for young women research workers appeared to be in industry. In 1977, for example, only 10.8% of all women doctoral scientists and engineers were employed by industry.

Evidence that the general employment prospects for young research scientists and engineers is likely to remain gloomy through most of the 1980s was presented by Mr Alan Fechter, head of scientific and technical personnel studies for the National Science Foundation.

This situation was created by a number of factors, such as the increased hiring of research workers into non-tenure-track positions, a general reduction of promotion opportunities, declining salaries relative to other sectors, and cutbacks in 2-year and marginal 4-year institutions as a result of falling student enrolments. □

Study shows only 1% real growth in basic research spending

THE combined effects of increasing inflation and Congressional budget-paring have meant that a strong push by the Carter administration for continued real growth in funding for basic research was successful "to only a limited degree" over the past year, according to a survey of actions taken on the 1980 budget prepared by the AAAS secretariat and published last week.

The survey points out that, as in previous years, Congress substantially increased the basic research budget of the National Institutes of Health but pared back the increases proposed for most other agencies.

"Excluding NIH, the total income increase in basic research over the fiscal year 1979 comes out at about 11%, compared to 13% proposed in the budget. With inflation approaching 10%, compared to less than 7% anticipated in the budget, not much real growth is left."

The survey points out that although the

administration failed to convince Congress to hold R & D funding for the NIH to the level appropriated for 1979, the rate of increase was substantially slowed from that enjoyed by NIH in recent years.

In contrast, although Congress had initially moved to reduce the proposed levels of expenditure for military research and development, as attention focused on SALT II and on Iran, legislators changed course and approved virtually the full amount recommended by the President.

"In general, Congress seems to be fully committed to providing full support to a large and expanding military R & D programme" says the survey. It points out that no major defence policy issues were fought out between Congress and the administration in the discussion of the military R & D budget.

The net effect of congressional actions, it concludes, was to raise support for basic research by one per cent. □

Carter plans 13% research boost

PRESIDENT Carter is to ask Congress for an increase of about 13% in financial support for basic science in the fiscal year 1981 — just enough to keep slightly ahead of expected inflation and to allow for real growth.

In his budget request, due to be presented to Congress on 28 January, the President is also expected to place particular emphasis on funding for engineering and the physical sciences, which have recently received less attention than the biomedical sciences.

The President gave a preview of his budget request on Monday at the presentation of 20 national medals of science, the highest honour awarded to US scientists and engineers by the federal government. He said that the net effect of his request will be to increase support for

basic science by 40% during the first three years of his administration — officials said later that the increase for the first two years was 24%.

The budget for the National Aeronautics and Space Administration is expected to reflect the impact of the additional costs of the space shuttle on space programmes. In particular, NASA officials have been unable to reverse an earlier decision by the Office of Management and Budget not to support research on an ion drive system which is essential if a joint mission is to be mounted to the comet Tempel 2 with a 'fly-by' of Halley's comet in 1985.

However, this year at least two projects are expected to be approved: the gamma ray observatory, and a national oceanographic satellite.

David Dickson

United Kingdom

Locust research cut

RESEARCH on locusts at the UK Centre for Overseas Pest Research is one of the casualties of recent cuts in government spending. For the past 50 years, the centre has combined research and field control and has been a major centre for visiting scientists from developing countries to do research and for the training young scientists.

With the cuts, all field work, research and training will stop and the locust experts employed by COPR will be available only as consultants. Within six to eight years, according to Dr Haskell, director of the centre, most of them will have left. The United Nations Food and Agriculture

Organisation in Rome has taken over the monitoring of potential areas of plague evolution and of organising international control measures but Dr Haskell fears that much of the centre's basic research on locusts will not be taken up elsewhere.

The centre's first director was Sir Boris Uvarov who determined that what had been thought of as two distinct species of locust were different forms of one species: the less innocuous 'grasshopper' and the plague-causing destroyer of crops. More recently, the centre has been renowned for its work on the relation of swarming to meteorological conditions and in establishing the importance of pheromones to swarm evolution.

Peter Collins

United Kingdom



Time running out for technology

FAR from making a smooth transition into the post-industrial world, Britain is heading for much greater competition, says a government report* published last week.

Britain must be prepared particularly for developments in "information technology and biotechnology, and of industries of high growth potential arising from the increasing need to conserve energy and materials" it says.

The booklet is the result of a working party, headed by Sir James Menter, of the UK's Advisory Council for Research and Development. ACARD called for the report principally to project the impact of potential technological change on employment, but the authors found it impossible to reach quantitative conclusions. But they argue "more unemployment results from loss of market share following a failure to innovate than from the introduction of new technology. Conversely, if new technology leads to an increase in market share there is generally an increase in employment opportunities".

The least predictable recommendations in the report are that the UK should consider setting up an agency to facilitate the import of new technologies from countries which have already taken a lead; and that large companies with R & D results that they do not intend to use should be encouraged to set up or seek out small firms better able to utilise such results.

The report suggests that "attempts by firms or industries in the UK to avoid competition from technologically more adept foreign competitors by specialising in certain (usually high-priced) sectors of the market (for example the motor cycle industry), by continuing to accept lower real wage rates and higher manning levels (car industry) are ultimately doomed to fail."

Robert Walgate

**Technological Change: threats and opportunities for the UK*, Cabinet Office, HMSO, £1.75

Italy

Health monitoring endangered at Séveso

THE lack of facilities available to local scientists for monitoring the health of people exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) at the Italian town of Séveso in July 1976 is endangering the health surveillance programme.

This is just one of the conclusions reported in the minutes of the International Steering Committee (ISC) of the Lombardy region's special office for Séveso. The ISC — composed in the main of internationally renowned scientists — was formed in the wake of the Séveso accident to consider the implications of exposure to dioxin by the local population.

To save the health surveillance programme, the ISC says it is of crucial importance for either local or national government to make a commitment to establish more permanent staffing within an epidemiological group set up specifically for the "Séveso follow-up". And in order to overcome the disillusion of local doctors in the current surveillance exercise, the ISC consider that one of the first priorities of the epidemiological group must be to enhance the motivation and to

increase the involvement of local medical practitioners and hospital staff in a long-term programme.

It is the view of the ISC that the skin disease chloracne is one of the best indications of exposure to dioxin. Individuals with chloracne, it says, represent a group "for whom biologically significant (as distinct from presumed) exposure [to dioxin] can be reasonably accepted". The ISC believes that it is this group which faces the greatest health risk and which should be monitored for many years, perhaps even for life. To assess the risks for this sector (a group composed entirely of children) it is suggested that two matching control groups — one not exposed to dioxin, and the other possibly exposed — should also be monitored.

Dioxin is known to be both a teratogen and a carcinogen in animals. Data on the danger it poses to the human fetus are simply not available. However, the ISC acknowledges that there may still be long-term risks to the fetus of "chronic exposure to very small amounts" of dioxin and the committee recommends that a registry be

kept of severe and minor congenital malformations; birth weight; prenatal and postnatal mortality rates; and the rate of spontaneous abortions.

An efficient cancer registry based on morbidity as well as mortality is also urgently required says the committee. Maintenance of such a registry is both difficult and time consuming, but the ISC points out that many countries carry out such programmes.

Over 1,000 individuals employed in the chemical industry are known to have been exposed to significant levels of dioxin over the last 30 years and the committee is anxious to obtain any information about their health problems. The ISC says that this information will help the Italian authorities to assess the adequacy of the health programmes for the Séveso area.

Failure to implement these proposals will mean that much information, important to the well being of the Séveso population, will be lost. If the ISC recommendations are ignored, many of the scientists on it could withdraw.

Alastair Hay

Eastern Europe

Scientific exchanges are needed for détente

SCIENTIFIC exchanges "agreed by treaty" should continue, irrespective of developments in Afghanistan, according to East German radio last week.

East Germany has a special interest in preserving *détente* at this stage — the first ever formal summit between Party Leader Erich Honecker and the West German Chancellor Helmut Schmidt is expected this spring. However, political spokespersons and commentators throughout the socialist bloc seem to be making a concerted effort to minimise the effect of the Soviet intervention in Afghanistan on *détente* in Europe.

Eastern hopes in this regard have undoubtedly been bolstered by the slow response of Western European governments in support of Carter's measures. The UK, for example, will hold a Cabinet Office meeting later this week to discuss British action (if any). An environmental working party is currently in Moscow, discussing odour prevention, under the auspices of the Intergovernmental Joint Commission for Trade and Technology. According to a representative of the Department of Trade, the meeting was probably allowed to continue because no member of the British delegation was of sufficiently high rank to warrant its cancellation at this stage.

Accordingly, while denying that the

Soviet Union has, in fact, done anything untoward in sending forces into Afghanistan, the socialist bloc commentators continue to urge *détente*. "Economic, scientific, technical and cultural links between countries", said Viktor Glazunov, one of Moscow Radio's chief "news analysts", "make up what you might call the material fabric of *détente*. A blow at the fabric strikes a blow at *détente* itself . . ."

The need to bolster *détente* is all the more urgent, with the Hamburg Scientific Forum only a month away. This forum is intended to be a meeting point for individual scientists to discuss problems in the context of East-West scientific cooperation, including contacts, communication and the exchange of information. Clearly world events, and the general issues of academic freedom and human rights are bound to be raised in that context.

Indeed, one human rights lobby, led by Valentin Turchin, and John Macdonald Q.C., would have suggested that western delegates should press for the forum to be postponed, until such time as the dissident physicist, Yuri Orlov, is released from strict regime labour camp. (The rationale of their argument being that Orlov, convicted in 1978 of slandering the Soviet state, had, in fact, been collecting material on alleged Soviet breaches of the Helsinki

accords, in his role as founder member of the clandestine Helsinki Monitoring Group in Moscow.)

Other action groups, while agreeing that the forum should take place, see it as a valuable platform for human rights. "In our opinion", says the Scientific and Medical Committee for Soviet Jewry, "for the exercise to be meaningful, not only should the Hamburg meeting include on its agenda the question of the freedom of the movement of scientists; also any scientific body must ensure that a clause dealing with this problem is written into its constitution."

This too is essentially the attitude taken by Bruce Kiernan, human rights coordinator of the American Association for the Advancement of Science, which, together with the Committee of Concerned Scientists will be holding a human rights' briefing for the US delegates to Hamburg early in February.

Those French scientists that are concerned with human rights have not yet made a formal statement; it is understood, however, that they will press for the guaranteeing of the personal security of scientists as an indispensable preliminary to any international cooperation — and that, the Helsinki accords being indivisible, it is impossible to dissociate "exchange programmes" from human rights.

Vera Rich

NEWS IN BRIEF

China issues new titles for engineers

CHINA'S State Council has issued temporary provisions for titles for the country's 21.5 million engineers and technicians. Issued on 28 December, the council's recommendations call for the titles, senior engineer, engineer, assistant engineer, senior technician, and experienced worker or technician to be granted according to previous contributions, vocational proficiency, previous schooling and seniority. The senior technician or experienced worker designations will be granted to those who have special skills, can solve key technical problems and have distinguished themselves in work. An editorial in the *Beijing Workers Daily* says the provisions "will help overcome the egalitarianist tendency that calls those who work more and better and those who work less and worse the same". Assessment will be done by special groups consisting mainly of experts. Party committees should guide the groups instead of interfering with their remit, the editorial says.

French unions refuse reactor safety report

FRENCH trade unions representing nuclear reactor workers have refused to accept the conclusions drawn in a safety report issued by the French electricity board. Released to the unions last week by the Director of Equipment, the 43 page report concludes that the cracks found in the tubular heat exchanger plates in the reactors, Tricastin I, Gravelines I and Dampierre I "do not constitute a risk to the workers or the population" and that "all the faults will be able to be monitored during the reactor's operation." The Communist trade union, the CGT, has demanded increased safeguards in the functioning of the safety valves in the primary cooling circuit. The socialist trade union, the CFDT, has objected to "numerous uncertainties in the security and safety of the installations" and the unaligned Force Ouvriere protested a lack of completeness in the report. "The politics of nuclear information is a reality and we cannot tolerate withholding information of any sort".

French Academy elects first woman to full membership

THE French Academy of Sciences has, for the first time in its 300 year history, elected a woman as a full member. She is Mme

Yvonne Choquet-Bruhat, a leading figure in the field of general relativity for 30 years, who has pioneered work on the Cauchy problem and the mathematical structure of Einstein's equations. The Academy, which admitted Pierre Curie and closed its doors to Marie Curie, has thus finally, if belatedly, recognised the contributions of women to knowledge. Speaking at the time of her election Mme Choquet-Bruhat said that she hoped she would not be the sole woman member of the Academy for long. Now that the breach has finally been made, it is expected that many more of France's talented women scientists will follow.

Jim Ritter

British nuclear reactors closed for cracks

TWO Magnox gas cooled reactors at Dungeness near Dover, have been closed for an indefinite period because of cracks in the expansion bellows of their primary cooling circuits. The cracks, up to a meter long, are located in the welds of the expansion units inserted into the 4 ft diameter cooling pipes. Originally discovered during the biennial routine inspection of reactor 1 in August 1978, the cracks were ruled to have been present during manufacture and not to have grown during the lifetime of the reactor. But a more detailed ultrasonic search on reactor 2 during its biennial inspection in April 1979, showed meter long cracks in many of the 24 bellows mountings. Reactor 2 is still shut down. The Central Electricity Generating Board, in conjunction with the Nuclear Installations Inspectorate then decided to close down reactor 1 for further tests last weekend. The CEBB will prepare a detailed safety report on the situation which must be passed by the NII before either reactor is restarted. According to the *Guardian*, engineers are reported as saying that the 16 year old reactors are unlikely to ever be used again.

British groups organise against transport of nuclear waste

THE London Regional Anti-Nuclear Alliance has announced a campaign to prevent the weekly shipment of three tons of nuclear waste from Dungeness to the Windscale reprocessing plant from passing through London. Citing US legislation which bans nuclear waste transport through New York City as a precedent, the Alliance says that the three previous derailments of trains carrying radioactive waste means that "it is only a matter of time before a serious accident occurs". According to the Alliance an accident damaging the flasks could cause

contamination within a 12 mile radius producing 8000 additional cancers and would render the area within a 3 mile radius uninhabitable for 70 years. The Alliance says that the flasks have not been tested to withstand impacts in excess of 30 miles per hour or fires lasting longer than 30 minutes and it calls for a rerouting of trains around London and other densely populated areas as well as the release of the government's evacuation plans in case of an accident.

Piperno still held without evidence

THE case of Italian physicist Franco Piperno, extradited to Italy from Paris last summer for "actions against the State" has entered a new phase. After 22 months of hearings and 20,000 pages of documents on the assassination of Aldo Moro, seven magistrates have recently published a 186 page report in Rome. The magistrates could find no evidence linking Piperno and his two colleagues in the Workers Autonomy Movement, Antonio Negri and Lanfranco Pace, with the assassination and have called for an additional inquiry. The report says that it "is extremely difficult to furnish proof, and because of the complexity of the accusation against them, the case of the three Autonomy leaders ought to be separated from that of the Red Brigades and a supplementary inquiry be effectuated". According to *Le Monde*, the prosecution believes it may find the evidence it needs from the recent testimony of an informer, Carlo Fioroni.

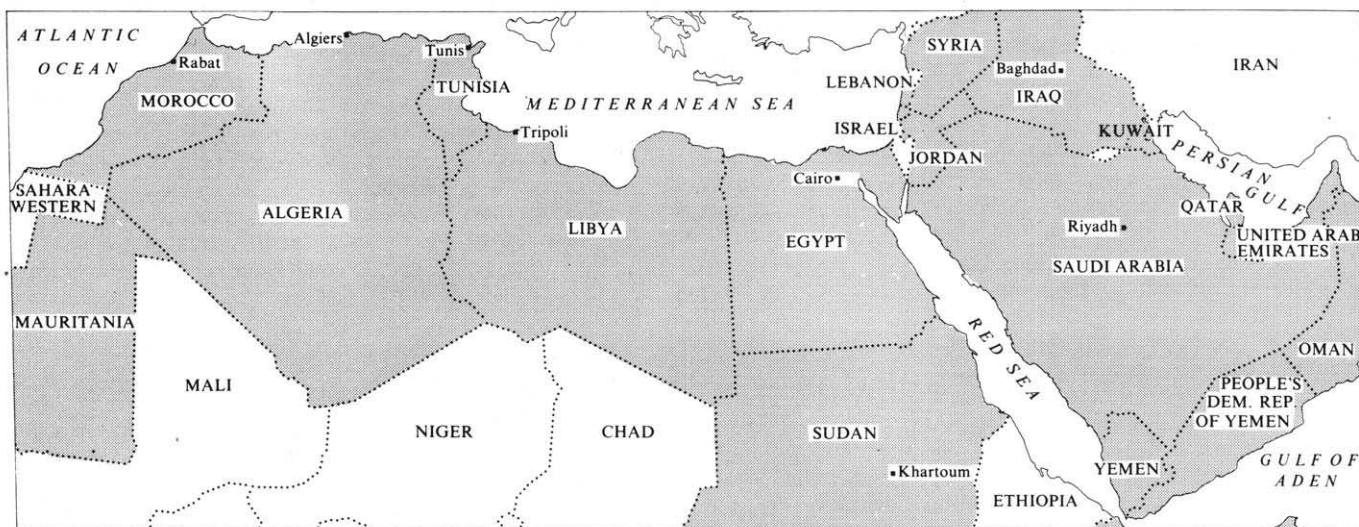
Bangladesh LANDSAT programme enters second phase

A THREE day international workshop on disaster preparation and remote sensing inaugurated the installation of an advanced ground station in Bangladesh for receiving satellite data on flood indications and major meteorological disturbances. Automatic data collection platforms will also be set up along the major rivers in Bangladesh to assist in water management and planning. Chaired by Dr B.M. Padya, Chief Technical Advisor of the World Meteorological Organisation, the conference was attended by representatives from Bangladesh, India and Thailand.

Satellite data has already been used in Bangladesh for land accretion and land use studies, changes in water reservoir levels and siltation surveys. Remote sensing has revealed desertification in northern districts and LANDSAT information has been used for afforestation projects, crop distribution studies, estimation of crop yields and salinity intrusion studies.

M. Kabir

FEATURES



Shaded countries are members of the League of Arab States. Somalia, not on the map, is also a member

Planning science in the Arab world

Many problems faced by scientists in the Arab world are common to all Third World countries. **Dr A B Zahlan** takes the Arab case to test some general assumptions about the problems of science and technology in developing countries

SCIENTIFIC research in the Arab world has been growing at a steady pace during the past three decades. Two thirds of this activity has been in the agricultural and medical fields where extensive institutions and qualified manpower now exist. The research work is generally of an applied nature and dwells on current problems. By contrast, research activity in all other fields is on a limited scale. In the basic sciences, for example, it does not account for more than 5% of publication output, and in areas of a vital developmental, industrial and defence nature — such as metallurgy, civil engineering, hydrocarbon chemistry, electronics, aerospace, — it is on a very small scale.

The fields of scientific research that are normally undertaken by university professors have been severely affected by the generally poor working conditions in the fifty or more Arab universities. Teaching loads are high, job security is low, libraries are poor or non-existent, laboratory and work-shop facilities are restrictive, administrative red tape is excessive, intellectual stimulation is

limited, standards for academic promotion are wanting, and technical and secretarial support staff remain scarce. These conditions of course do not prevail at the same level in all institutions, and an active research programme often flourishes in difficult circumstances thanks to the energies of a few researchers. Moreover, a small number of scientists have created permanent links with research groups in Europe or the US.

Various statistical compilations set a figure of about 40,000 for the number of researchers in Arab countries. These staff more than 500 R&D institutions and 50 universities, the latter enrolling more than 1 million students. However, the constraints on their capabilities have reduced their contributions to the scientific literature to a meagre 1,500 per annum. The absence of adequate publications in the applied sciences at one stroke limits the development of appropriate teaching materials and condemns workers to researching and rediscovering similar problems. The present level of funding in the range of \$1-2 per capita per year during the 1970s has, of course, also been a constraining factor.

Despite these drawbacks, the scientific communities in the Arab world have accumulated considerable experience and have experimented with a broad range of ideas and instruments. Many of the

problems faced by Arab researchers are common to Third World countries, and the Arab experience thus has a universal value.

The concepts underlying much of current thinking in science and technology in developing countries emphasise the need for high level manpower, the integration of research activities in science and technology with development plans, the central planning and management of the activity, the linking of the central management, (often called 'science policy making body', SPMB) to the highest decision maker, and so on. These notions are so reasonable that very little in the way of empirical testing has been attempted. The Arab experience in the use and application of these notions has made it possible to evaluate these concepts in real-life situations.

One finds, for example, that science policy making bodies still subscribe to the belief that the shortage of scientific manpower is an obstacle to the development of research programmes. However, the expansion of the secondary and university educational systems in the Arab world as well as the access to foreign educational systems (the USSR, eastern and western countries, and North America) have combined to eliminate the supply of high level manpower as the determining factor in scientific research or in any other function where such manpower is needed. Thus, although the Lebanese National Council for Scientific Research (NCSR) subscribed to the principle that the formation of scientific manpower was a necessary step prior to the funding of research projects, only 10% of its own grants went to recipients of NCSR

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advanced study fellowships; and the recipients of the remaining 90% of the grants had not required NCSR support for their higher education, having completed their advanced education before the NCSR adopted its manpower formation policy.

The feasibility of centrally planned scientific activity has been taken for granted by developing countries, and especially by UNESCO's Science Policy Division. Egypt has been committed to such an approach. More than eight major changes in its SPMB's during the past two decades clearly undermines the effectiveness of central planning. The Science Council (established in 1956) created a system of committees for the planning and management of scientific activity. Between 1958 and 1960, it mobilised 3,000 Egyptian scientists to prepare a five-year plan. This effort was sidetracked, however, in 1961 by the establishment of the Ministry of Scientific Research. There were further changes in 1964, 1965, 1968, 1971, 1975 and finally in 1976.

Since 1956 the councils and committees established to plan and manage scientific activity have grown in size and number. By 1977 the Academy of Scientific Research and Technology had 17 research councils and 97 subject committees involving more than 1,300 members for planning and managing its programmes. Of the 97 subject committees only a third held one or more meetings attended by 50% or more of their committee members; and 53% held no meetings. A detailed analysis revealed that there was no correlation between the number of meetings held and the amount of funds appropriated, or between the declared priorities of the Academy's science policy and the activities of the committees or councils or the funds appropriated. Although a basic objective of the Academy is to concentrate on the solution of urgent problems, only 5.6% of 1977 appropriations were committed to projects whose duration was two years or less, and 51% were granted to projects whose duration was five to ten years.

The linkage of the SPMB to the decision-makers has posed a number of interesting problems. UNESCO, for example, advocates that this linkage be to the highest decision maker in the country in order that financial support be guaranteed and that the integration between development planning and science and technology be efficiently effected. Despite the importance given to this linkage in the international literature on the subject, one finds surprisingly little concern for the actual behaviour of these Third World institutions. Since SPMB's are central to the relationship between the scientific community and the decision maker, their stability is vital to the development of effective personal and institutional relationships. The eight major changes in the SPMB in Egypt noted earlier reduce the credibility of such an assumption; such a

high level of discontinuity in the institutional framework undermines both the linkage to the decision maker and the human and social relationships within the community itself. In addition to institutional instabilities one must also consider the frequent changes of the officials responsible for the SPMB.

The situation on the decision-maker's side is no less incoherent. Frequent changes in ministers and in policies also occur. Civil disturbances and economic crises take their toll of scientific activity. The first year of the Lebanese civil war, for example, reduced the output of the scientific community there by more than 42%.

Much of the international discourse on science and technology in the contexts of UNCSAT and the UN conference on science and technology for development revolved around the notion of "applying" science and technology to the development process. Yet the major obstacles to achieving such a useful objective were left out of most of the deliberations. These obstacles are due to the structure of the planning process, the inadequate communications between the scientific community and planners, bureaucratic resistance to data collection and dissemination, and the domination of the central planning process by economic, political and financial considerations. The widespread utilisation of the turnkey type of contracts with foreign firms reduces opportunities for applying indigenous science and technology considerably. The character of government operations is important, rather than the institutional link of the SPMB to central authority. As a matter of fact, it does not seem to matter whether the linkage is to the head of state or to the minister of higher education and scientific research.

Most of the Arab countries have now reached a phase where the major obstacles to further progress lie in the weaknesses of the existing institutions, the planning practices of ministries, the means available to university professors to undertake scientific research, and cultural policies.

The international connection

The scientific communities and institutions of the Arab world have evolved a complex set of relationships with foreign countries. To begin with, most scientific manpower engaged in research have relationships with scientists in Europe, the USSR or US. A number of scientific unions already exist in the Arab states and many of these are members of the International Council of Scientific Unions (ICSU). Egyptian scientific councils belong to most of the ICSU councils, and research councils in roughly half the Arab states are members in one or more of the ICSU councils.

Numerous research institutions in the Arab world have been established through bilateral agreements. The nuclear research

centres in Baghdad and Cairo, and the bilharziasis research institute in Cairo were established by the USSR and Federal Republic of Germany respectively. In 1942, the US Navy established in Cairo the US Naval Medical Research Unit Number Three (NAMRU-3) which by 1977 had grown to 26 military personnel and 206 civilians (203 from Egypt). NAMRU-3 has played an active role in medical research in Egypt. In 1976, the US and Saudi Arabia signed an agreement through which the US is to assist Saudi Arabia to establish the Saudi Arabian National Centre for Science and Technology (SANCST). The US and Saudi Arabia also have a joint five year \$100 million research programme in solar energy.

UN agencies have been involved in a number of different ways in the application of science and technology in developing countries. UN support to scientific research activity, or to the strengthening of indigenous technological capabilities of Third World countries, consumes no more than 1.5% of the \$3 billion UN budget. The Arab world share of these \$45 million is of the order of \$1-2 million.

The number of scientific and technical meetings in the Arab world has been steadily increasing. The Cultural Department of the League of Arab States sponsored these meetings in the fifties. Since then the Arab League Educational Cultural and Scientific Organisation (ALECSO) has been instrumental in convening them. The growing number of scientific associations and societies have also been actively organising scientific meetings.

Likely trends

On the national and regional level, a large number of small scale but steady changes have been registered. New institutions and new universities are created annually. The position that Egypt (with 25% of the population of the Arab world) held in 1950 with virtually the only Arab centre for scientific work (more than 80% of Arab publications in 1950 were by Egyptians) has been changing steadily. In 1967 Egypt produced 63%, and in 1976 55%, of Arab publications; by the year 2000 Egypt's share will be below 40%. This reduction is not due to a decrease of activity in Egypt but rather to an expansion in the other Arab states. For example, the combined output of Kuwait, Libya and Iraq (11% of Arab world population) which was 8% in 1967 increased to 14% in 1976. It is likely that the share of these three countries would have increased to 30% in the year 2000.

The internal movement of scientific manpower between Arab states has been steadily increasing. This has made the development of social and educational services in Arab oil producing countries at a high rate possible. During the past decade

most of the increases in the allocations for R&D in the Arab world have taken place in oil producing countries; elsewhere, little increase in funding is recorded.

The Arab scientific communities, the educational systems, their immediate environments, their relationships to the users of technology, and the resources of the Arab states have all been undergoing far-reaching changes. It is very difficult at this moment to identify clearly the new directions that are emerging. On the surface, it would seem that the traditions of the past three decades are firmly entrenched. This can be seen from the CASTARAB 1976 meeting in Rabat, the 1978 Kuwait Fund for Arab Economic Development feasibility study on the proposed Arab Fund for Scientific and Technological Development, as well as the national papers submitted to UNCSTD. Nevertheless a large number of indicators point to the possibility of major departures. Since the early fifties, the production of scientific and technical manpower has been expanding at an exponential rate, with a doubling time of about 5.3 years. If this rate continues, the one million university graduates of 1978 would have grown to more than 12 million by the year 2000. The 24,000 foreign trained doctorate holders could reach a level of 150,000–250,000 by the year 2000.

Meanwhile, the capacity of the US and Europe to drain unwanted high level manpower has been reduced by the economic recession registered there. Furthermore, the size of Arab technical manpower is rapidly reaching a level that makes likely drains an insignificant portion of the total pool of educated manpower. The absorption of this enormous manpower cannot be achieved without effecting major structural changes in current practices of planning and executing development projects. All Arab countries are undergoing rapid social and economic change. Ministries, institutions, industrial installations, and transport systems are all being developed at a high rate. But many developmental projects are being executed in a manner that does not utilise existing technical skills or institutions. This leads to a low level of employment generation within the Arab countries themselves. An interesting comparison provides an indication of the extent of this fact: Imperial Chemical Industries (ICI) provides direct and indirect employment in the UK comparable to that of the hydrocarbon sector in the Arab world; but the sales of Arab oil are roughly ten times those of ICI. Obviously the only way in which the five million graduates in the applied and basic sciences are going to find employment is if the present technological dependence is reduced through the use of indigenous firms and institutions. Technology policies will certainly assume increasing social and political importance during the next two decades. The very stability of Arab countries will depend on

Mountains out of molehills

ONE of the less attractive habits of our species, *Homo sapiens* (sic), is the way in which we attribute our unpleasant behaviour patterns to other animals. Thus the human glutton is described as a pig or a hog, and the term swine is used to cover a multitude of our sins. Male chauvinists are pigs, and their women bitches when they assert their rights, cows when they do not, and cats when they criticise other women. Occasionally diminutives like kitten are used more affectionately, but as a rule the comparison with an animal is a derogatory one.

1979 has been called 'The Year of the Mole' — with the mole cast as the villain of the piece. Thus while the majority of viewers could not make head or tail of the BBC television serial *Tinker, Tailor, Soldier, Spy*, they did understand that there was a traitor high up in the security organisation who was passing vital information to the enemy — in other words, a 'mole'. This programme had hardly left our screens when we had the real life case of Anthony Blunt, a distinguished art historian and advisor on pictures to the Queen, who confessed to having been a Communist agent passing information to the Russians when he was attached to the British secret service. He was the mole *par excellence*. Then for some years British Leyland, the ailing motor manufacturer, has had its troubles exacerbated by the actions of a Communist shop steward, commonly called the 'red mole'.

Yet the furry little insectivore *Talpa europaea*, has little in common with a spy or a disruptive agitator. It must be admitted that it is equally unlike the loveable and friendly animal featured in Kenneth Grahame's delightful children's book *The Wind in the Willows*, and presented so vividly on stage in London each Christmas by the redoubtable octogenarian actor Mr Richard Goolden in the play *Toad of Toad Hall*. Moles do indeed live underground in burrows, but their most striking characteristic is their solitariness. Rudyard Kipling wrote, in this case with reasonable accuracy, about 'The Cat which Walked Alone', but the mole is very much more independent than



KENNETH MELLANBY

the most self-centred domestic pussy.

In fact it is difficult to see anything in the life history of the mole in common with that of a spy. For almost the whole of their lives, both males and females occupy their own separate sections of underground tunnelling, and spend the time in sleep or in running up and down picking up worms and insects which have dropped into the burrow. Should a mole be foolish enough to invade another's territory, it is usually driven off at once. Except for a brief period once a year when the female accepts the attentions of the male, when mole meets mole there is at once open hostility and even bloody combat. There is nothing subtle, deceitful or underhand in the behaviour of *Talpa*.

Gardeners who suffer from the appearance of molehills on their carefully manicured lawns may perhaps be excused for saying nasty things about the culprit, but at least they must admit that by making its presence so noticeable the burrower is hardly being secretive or underhand. So should we not try to find some other zoological sobriquet for a traitor or an industrial troublemaker? I fear that any suggestions will arise the wrath of some other mammalogist involved with the libelled species.

Finally, I find that I am, unwittingly, involved in an attempt to cash in on the current interest in moles. I read in the London newspaper *The Financial Times* that: "Collins the publishers tell me that they have had some discussions about expanding the title of Professor Kenneth Mellanby's book *The Mole to The Mole — the Blunt Truth*".

the development of employment-generating technology policies.

A profound religious and ethical rejection of the stark contrast between wealth and poverty pervades the Arab world. This has so far led to the establishment of a number of Arab funds (national and regional) that aim to assist sister countries in their development. Finally, the difficulties and mechanics of development are becoming clearer, the

social and political cost of inaction is growing increasingly evident, the enormous resources of the Arab world are gradually coming under the control of local institutions and governments. What, when and how a zipper action will operate to cohere all of these disjointed resources is difficult to forecast. But when it does happen the scientific communities of the Arab world will be called on to play a leading role. □

CORRESPONDENCE

Dismissal of Professor Scheer was justified

SIR, — As a member of the federal state government of Bremen I would like to make the following remarks to correct the letter from Uwe Lahl and W. Thiemann of Bremen University (27 September, page 252).

The Senate of the Freie Hansestadt Bremen, the federal state government, has not pursued the dismissal of Professor Jens Scheer from the Bremen civil service because of his political and scientific activities involved with his criticism of the nuclear politics of the Federal Republic of Germany. The Freie Hansestadt Bremen, even the Senate itself, is in fact taking a decidedly critical position regarding the nuclear politics of the Federal Republic of Germany.

Nor has the Bremen government pursued the dismissal of Professor Scheer primarily because he belongs to a non-prohibited political party (the KPD; the Communist Party of Germany). The real reason which has tipped the scales towards disciplinary action against Professor Scheer is rather as follows.

Professor Scheer advocates in public and in Bremen University, while practicing his public duties and abusing his official position, the removal of our free democratic constitutional state of the Federal Republic by violence, and the erection of the dictatorship of the proletariat in its place; this in reality would be the dictatorship of a small elite. In making this aim (of violence) the declared object of his public service activities he abuses the official position given to him.

By the way Professor Scheer has advocated the use of violence not merely verbally, he has already been sentenced for taking part in violence against persons dissenting from his political opinion.

Therefore one cannot reasonably talk about the Bremen State trying to bring scientists under political control by this disciplinary action. Professor Scheer can make use of his constitutional rights in every allowable way. But he cannot legally claim the violent destruction of this state, and at the same time accept payment by the state. All civil rights remain guaranteed to Professor Scheer as a citizen and a scientist of this republic.

The Bremen state government is anxious to learn from German history. The first German Republic of Weimar allowed civil servants to challenge the state which they were sworn to serve, and in the end even to bring about its ruin. The Federal Republic of Germany and especially the German Social Democrats to which I belong have learned their lesson from Weimar.

Finally, the statement "He was not allowed a complete defence" is spun out of thin air. He and his lawyer had every opportunity to plead their position, and they did use it.

Yours faithfully,

FRANKE

Freie Hansestadt
Bremen, FRG

The Sirius Mystery

SIR, — Readers of *Nature* may well be aware from the review of my book *The Sirius Mystery* in the issue of 17 June, 1976 (pages 617–8), that "the problem of the remarkable knowledge of the Dogon people of Mali", referred to by Michael Rowan-Robinson in his review of Carl Sagan's *Broca's Brain* (8 November, 1979, pages 176–7), was first brought to public attention by myself. Although in his review Rowan-Robinson does not mention me by name, and in his book

Sagan barely refers to my seminal position with regard to public knowledge of this material except in passing, anyone truly interested in this subject is perfectly aware that they are referring to myself when discussing this matter.

It would be inappropriate for me to discuss Sagan's own errors here. But I would like to reply to statements made by Rowan-Robinson, who says that "Sagan has performed a useful service in collecting together examples of public gullibility and demonstrating how a mixture of deliberate fraud and uncritical thinking has allowed millions to be bamboozled . . .". He says this immediately after discussing my material.

I strongly object to the inference that I have been guilty of fraud in bamboozling a gullible public, and as for "uncritical thinking", the many serious and thoughtful reviews of my book in this very journal as well as others of distinction testify to the fact that although the implications of my work have aroused controversy, it is not widely thought, nor do I obviously think myself, that "uncritical thinking" is a fault of which I have been obviously guilty, especially as my conclusions were all explicitly hypothetical.

The anthropologists who have spent the 48 years from 1931 living with the Dogon tribe are agreed that the knowledge of the Sirius system which they possessed could not have come from Europeans, who in any case had themselves only discovered the superdense nature of Sirius B in about 1926. Knowledge of Sirius B amongst the Dogon goes back hundreds of years, as shown by physical evidence, some of which was even held up in front of television cameras by Dr Germaine Dieterlen, who insisted that it was 400 years old, and said that the European contact theory (espoused by Sagan) was "absurd".

But I do not wish to open this controversy again here. It is in the context of saying that Sagan has shown that the Dogon information "could all have been learnt from contacts with Europeans" (which is untrue) that Rowan-Robinson does me less than justice by saying this is better than it having "been learnt from little green men in UFOs". I have never at any time suggested this; I went out of my way in my book to say that I do not believe that UFOs are extraterrestrial craft of any kind, or connected with my subject, and Rowan-Robinson's remarks are therefore a slur against my intelligence and quality of work which I most strongly resent.

Rowan-Robinson also betrays an indefensible bias against the serious possibility that our planet has at some time in its history been visited by intelligent extraterrestrials. What is so remarkable about this *per se*? Are we so incapable of speculating about these matters that we deny them solely because such a visitation seems to us extraordinary? The electric light bulb would have seemed extraordinary to Plato and Aristotle, but does that rule out the possibility of its eventual invention? This is the kind of inverted logic demonstrated by those whose minds are too small for large notions.

Yours faithfully

ROBERT K.G. TEMPLE

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Standardisation of reference citation

SIR, — A significant part of a scientist's time is spent in drafting manuscripts for publication. Part of this task involves the

careful listing and checking of references. At present, most journals have their own distinctive style and even basic references have to be rearranged with considerable waste of time and effort as every new paper is drafted. Some idea of the variability of reference citation can be seen if the bibliographies of the following internationally recognised marine science journals (first published in sequence between 1953 and 1979) are examined: *Deep-Sea Res.*, *Mar. Biol.*, *J. exp. mar. Biol. Ecol.*, *Estuar. Cst Mar. Sci.*, *Oceanol. Acta.*, *J. Plankton Res.* One conclusion that can be drawn from such an examination is that the variability of reference citation is increasing with time.

Some progress has been made towards international standardisation with the introduction of the following international standards: ISO 4-1972 (E), Documentation — International Code for the Abbreviation of Titles of Periodicals and ISO 833-1974, Documentation — International List of Periodical Title Word Abbreviations. (See also BS 4148 Part 1: 1970 and BS 4148 Part 2: 1975.) The establishment of this international standard for over five years does not seem to be acknowledged by many editors (including *Nature*'s) who still recommend the fourth edition of the *World List of Scientific Publications* for journal abbreviations, a list which was last published in 1963 to 1965! In the absence of a properly updated world list and prior to the publication of the new international standard, some journals (such as *Journal of the Marine Biological Association of the United Kingdom*) have reverted to printing the full titles of journals.

There is so far no international agreement on bibliographic references, rather the opposite, as the American National Standard ANSI Z39.29-1977 and British Standards BS 1629:1976 and BS 5604:1978 differ considerably from each other in their recommendations and from each of the journals cited above. Major differences between the two national standards are the use of capitals, italics and bold face in the British Standard in contrast to a single type face and more complicated punctuation rules in the American standard. The 'Harvard' system with the year of publication following the author and a form of the 'Numeric' system as used in *Nature* are the two formats recommended in the new British Standards BS 5605:1978 which was specifically prepared for editors and authors of articles in journals and books. The 'Harvard' format comes closest to that now used by most journals. The simpler punctuation of this standard is also preferable, although the adoption of a simple typeface as used in the American system may be more compatible with computerisation of references.

A standard for abbreviated references, as used for instance in *Nature*, is only given in ANSI Z39.29-1977 restricting information to the journal title, volume number, pages and year of publication. This is not sufficient for scientific journals as one needs to assess immediately the 'pedigree' of a particular piece of work by reference to the original authors and if possible an abbreviated title.

Publication of an international standard for bibliographic references in scientific journals is overdue. Major publishing houses, editors of journals and scientists should be consulted in this standardisation process as well as librarians and national standardisation authorities.

Yours faithfully,

P.C. REID

Institute for Marine Environmental Research,
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NEWS AND VIEWS

Pain, enkephalin and acupuncture

from Shin-Ho Chung and Anthony Dickenson

DURING the past few years, there have been enormous strides in understanding the neural mechanisms underlying the perception of pain. From what we now know, it is easy to see why the mechanism of pain perception has remained enigmatic for so long. Unlike the other senses, there seem to be no cortical centres which are solely devoted to processing pain, nor is there a discrete fibre bundle, which can be severed to alleviate chronic pain. Indeed, the system seems to be elaborately designed to ensure that it is fail-safe, with an added feedback loop which, when activated, reduces the intensity of persistent pain.

It is now firmly established that small-diameter fibres, whose endings are distributed throughout the body, including skin, muscles and viscera, respond specifically to painful stimuli. These fibres, on entering the spinal cord, branch profusely and form synapses in the superficial layers of the dorsal horn, mainly in the marginal zone and the substantia gelatinosa¹. Here the fine terminals of axons and dendrites, together with collaterals of ascending and descending fibre tracts, form a dense jungle of neuropil, the precise wiring diagram of which has yet to be elucidated. There are two distinct groups of spinal neurones which respond to painful stimuli, one located in the superficial layer (lamina I) and involved mainly in transmitting sharp pain, and the other in the deeper layers of the dorsal horn (laminae IV and V) responding to intense pain². Through a dense meshwork of short and long axons, these cells transmit messages further centrally. Some of these axons terminate within the spinal cord³, whereas others ascend to the brainstem reticular nuclei⁴. The longest bundle proceeds to the thalamus^{5,6}, accompanied by other axons arising from the brainstem. In stark contrast to the other senses, the neural organisation of the pain system is characterised by elaborate feedback

networks. Terminals of at least three descending fibre tracts, which originate from the brainstem nuclei, form synapses in the dorsal horn⁷. In addition numerous short axons of spinal neurones traverse a few segments, their terminals interlacing with the dendrites of pain cells located further rostrally¹.

The interplay between the incoming pain messages and the descending influence from the brainstem has recently been the focus of intensive investigation. In 1964 Taub⁸ first discovered that spinal neurones responding to painful stimuli could be silenced when certain areas of the brainstem were stimulated electrically. This initial finding of descending inhibitory pathways, confirmed subsequently by others, has recently been extended. The physiological effect on spinal neurones of electrical stimulation of the brainstem is now known to be confined to cells responding to pain, especially those in laminae IV and V⁹⁻¹¹; other neurones nearby which convey, for example, information concerning touch or temperature, are uninfluenced by this manoeuvre. Careful mapping has revealed that there are discrete nuclei in the brainstem, stimulation of which produces potent neuronal inhibition and behavioural analgesic effects; among these are the nucleus raphe magnus and the periaqueductal grey¹⁰⁻¹³. The raphe magnus is known to be the origin of certain descending fibre tracts^{14,15}, but the periaqueductal grey, which is located along the length of the aqueduct, seems to exert its influence indirectly on the dorsal horn through the nucleus raphe magnus^{16,17}.

The analgesic effect of morphine and related compounds can now be understood in terms of their inhibitory influence on the transmission of pain. For reasons which are not yet entirely clear, morphine reduces the efficiency of synaptic transmission between pain fibres entering the spinal cord and their target cells in the dorsal horn¹⁸⁻²⁰. A small quantity of morphine injected into the substantia gelatinosa reduces sensitivity to pain, when tested either behaviourally²¹ or electrophysiologically²². The action of morphine, however, is also mediated by the

descending inhibitory system of the brainstem. From numerous studies involving gross lesions, microinjection of morphine, and biochemical mapping of the distribution of opiate receptors, the same conclusion emerges; the cells in the periaqueductal grey and the nucleus raphe magnus, richly endowed with opiate receptors, detect the presence of morphine and transmit this message down to the dorsal horn of the spinal cord^{23,24}. Here the terminals of the descending fibres release serotonin²⁵, which, through mechanisms yet to be discovered, interferes with the excitatory synaptic processes between the primary pain fibres and spinal neurones²⁶. This inhibitory modulation on the transmission of pain, the essence of Melzack and Wall's 'Gate Theory'²⁷, relies entirely on the integrity of the brainstem descending system. If, for example, the raphe magnus or the periaqueductal grey are destroyed, or naloxone (a specific opiate antagonist) is injected into these brain areas, then the analgesic effect of morphine is drastically reduced^{23,28-30}. Moreover, both the neuronal inhibition and the behavioural analgesia induced by electrical stimulation of the raphe magnus are reduced by naloxone^{31,32}. Why, of all other substances, opiates and opiates alone turn out to be such potent pain-killers is one of the deep mysteries of the brain.

While the neural basis for analgesia was beginning to be understood, two related pentapeptides, known as Met-enkephalin and Leu-enkephalin, were isolated from the brain. These opioid substances, along with other related peptides containing the enkephalin sequence, are present in axonal endings of both central and peripheral nerves. They bear a stereochemical resemblance to opiates and are known to be synthesised locally. Biochemical and immunohistochemical analyses have revealed that those parts of the nervous system directly involved in mediating or modulating pain are especially rich in both opiate receptors and the endogenous opioid substances. Since these discoveries, some of the electrophysiological and behavioural findings obtained with morphine and electrical stimulation of the brainstem have been replicated with

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enkephalins^{33,34}. All the findings to date show that the action of morphine can be mimicked by enkephalins. Some problems remain however. We want to know, for example, where on the neuronal membrane opiate receptors are located and how the peptides influence the receptors to generate synaptic currents.

If the brainstem inhibitory nuclei are activated by naturally occurring opiates as a result of injury, would it not be possible to mimic this by stimulating peripheral nerve fibres carrying pain messages and so reduce the intensity of persistent pain? The technique of acupuncture, which the Chinese have traditionally used for combating pain, seems to be based on this principle. In a series of elegant studies, Le Bars and his colleagues^{35,36} have demonstrated that cells in lamina V of the spinal cord responding steadily to painful stimuli applied to the hind limb can be silenced by activating, either with natural or electrical stimulation, pain fibres from other parts of the body. These lamina V cells are the ones that respond to intense pain, and the reduction of their activity is brought about by the descending inhibitory system from the brainstem and also can be mediated by opiates³⁷. On the other hand, the activity of some cells can be inhibited by the local circuitry within the spinal cord^{27,38}, without involving the brainstem.

Thus, it is now becoming clear that the analgesic effect of acupuncture is the result of interaction between impulses from the origin of pain and afferent impulses from the acupuncture point³⁹, and that this interaction takes place at different levels of

the central nervous system⁴⁰. For acupuncture to be effective in relieving pain, however, the involvement of the nucleus raphe magnus and endogenous opiates is required. There are several independent lines of evidence for this assertion. Pomeranz and his colleagues^{41,42} have shown that the analgesic effect produced by acupuncture in animals is abolished if naloxone is injected. Several clinical studies also note that strong acupuncture is most effective for alleviating chronic pain in humans⁴³⁻⁴⁶, and the analgesia so produced is antagonised by injection of naloxone^{43,45,47}. In retrospect, it is clear that the Chinese have been at the forefront of research into pain. They have known for several years, that the nucleus raphe magnus⁴⁸, the serotonergic system⁴⁹, and inhibitions of lamina V cells⁵⁰ are

intimately involved in producing the analgesic effect of acupuncture. To the Western mind, it has been inconceivable until now, exactly how placing a single needle into a certain point of the body could produce such a potent amelioration of pain elsewhere in the body.

There is a glimmer of hope that, in the near future, new methods of controlling pain and new analgesics without addictive properties may be found. But the fundamental question of how we perceive pain still remains to be answered. Pain, after all, is a conscious awareness, and so involves the full participation of higher cortical centres. On the role of the cortex in perceiving pain, we remain totally ignorant. It is towards the elucidation of the interaction between perception and transmission of pain that future research must be directed. □

Footprints of the Sun

from Bevan M. French

WHAT has the Sun been up to lately? The question is becoming important both scientifically and socially, as more evidence appears that the Sun is (and has been) a variable star. Astronomers worry about the Sun's variability as a clue to understanding stellar behaviour. Everyone else is (or should be) concerned about what future solar variations might do to our weather, climate, food production, and civilisation. Understanding the Sun's past may help us predict its future behaviour a little better.

Where are the records of the Sun's past? Almost everywhere in the Solar System, if the results presented at the Conference on the Ancient Sun last October*, are any indication. Traces of solar behaviour have been left on many worlds, and the list of materials that hold assorted obscure parts of the record reads like a planetary jumble sale: historical documents, tree rings, pollen collections, deep-sea sediments, glacial deposits, spacecraft measurements, Marine and Viking photographs of Mars, polar ice cores, Apollo lunar samples, and meteorites. In each material the solar record, while readable, is often incomplete, ambiguous, or model-dependent. A greater problem in putting the pieces together is the lack of communication between the unusually wide range of specialists involved — solar astronomers, historians, nuclear physicists, glaciologists, tree-ring daters, and the geologists, geochemists, and physicists involved in moon rock and meteorite studies.

The goal of the Conference was communication: to introduce each

participant to the data available from other fields. It succeeded extremely well. Solar astronomers described for geologists the current state of theories (or the lack thereof) about how the Sun works. Others reviewed the information about solar variability that has been extracted from terrestrial climate records, from historical records, and from lunar samples and meteorites. These reviews set the stage for detailed examinations of solar variability over three timescales: short-term (0–10² yr), intermediate (10²–10⁵ yr), and long-term (10⁵–10⁹ yr).

There is clear evidence of short-term variability in the present-day Sun, even though detailed records of such things as solar flare activity and the solar constant (*S*) go back less than 50 years and reliable sunspot records cover less than two centuries. Short-term (hours to days) changes are observed in the solar radio, ultraviolet, and X-ray outputs (W.J. Borucki, NASA-AMES Research Center; P.V. Foukal, AER Inc., Cambridge, Massachusetts), and in the flux, velocity, and He/H ratio in the solar wind (A.J. Hundhausen, HAO, Boulder). Variations in the composition of solar flare particles have been measured by spacecraft (R.A. Mewaldt, Caltech, Pasadena), and measurements of lunar samples indicate significant variations in the proton flux on the order of decades (R.C. Reedy, Los Alamos Scientific Laboratory). The modulation of galactic cosmic rays by the 11-yr-cycle of the solar activity has been known for some time, but studies of cosmogenic radioactivities in meteorites that fell during the period 1967–78 (J.C. Evans, Johnson Space Center, Houston) indicates that the modulation is about three times as intense as expected. Despite these variations, the solar constant (*S*) has

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*Held at Boulder, Colorado on 16–19 October 1979. A limited number of abstract volumes are available from: Administrative Office, Lunar and Planetary Institute, Houston, Texas 77058 USA. US requests should include \$2.00 to cover handling and mailing costs. Non-US requests should include \$1.05 for return by surface mail, \$4.25 for airmail return. The Proceedings of the Conference will be published in late 1980 by Pergamon Press.

apparently remained constant to within about 1%, but recent spacecraft measurements suggest that variations of a few tenths of a percent have actually been detected. Even such small changes have significant effects on the Earth's climate.

In the intermediate period (10^2 – 10^5 yr), historical records become spotty, and scientists are dependent on the solar record in natural materials (both terrestrial and extraterrestrial). The Maunder Minimum, a period of reduced solar activity (1645–1715 AD) characterised by a virtual absence of sunspots, has now been well established by a combination of historical records (J.A. Eddy, HAO, Boulder) and ^{14}C studies of bristlecone pine tree rings (M. Stuiver, University of Washington, Seattle); the effects of reduced solar activity at this time may also have been detected in iron meteorites (O.A. Schaeffer, SUNY, Stony Brook). Earlier periods of both reduced and increased solar activity are also seen in the bristlecone pine records back to about 5,000 b.p. (Stuiver).

A debate about possible variations in solar flare intensity during the past 10^4 years has been generated by the record of solar flare particle tracks preserved in rocks and small crystals collected from the surface of the Moon. Comparative counts of solar flare tracks and the microcraters formed by cosmic dust particles suggest that both the dust flux and flare activity have been constant for about 5×10^5 yr (E. Zinner, Washington University, St Louis), but the possibility of higher flare activity about 10^4 years ago is still being considered (H. Zook, NASA Johnson Space Center, Houston). One suggestion of higher flare activity is the excess amount of ^{14}C detected in lunar surface soils (E.L. Fireman, Smithsonian Astrophysical Observatory). This could have been produced by solar flares. The alternative explanation, that the ^{14}C was produced in the Sun and implanted by the solar wind (Fireman), raises theoretical problems about just how ^{14}C is now being made inside the Sun.

Long-term variability (10^5 – 10^9 yr) involves the problem of what the 'early Sun' was like soon after its formation. Until about a decade ago, this problem area was inhabited entirely by theorists. Now a new group of scientists is extracting information (and even solar particles) from samples that actually saw the face of the ancient Sun — rock breccias from the lunar highlands and tiny crystals that were compacted into meteorites more than 4×10^9 years ago.

To quote James Arnold's (University of California, San Diego) summary of the Ancient Sun: "Things haven't changed an awful lot." G. Crozaz' (Washington University, St Louis) review made the

following points: (1) solar flares were active at least 4.2×10^9 years ago and left particle tracks in meteorite grains exposed to the Sun at that time; (2) the solar flare energy spectrum (measured in lunar samples, meteorites, and a glass lens from the Surveyor 3 spacecraft) seems to have been about the same for the last 4×10^9 yr; (3) enrichment of heavy elements at low energies (<10 MeV) is seen in both ancient and modern flares; (4) no convincing evidence for higher solar activity during the early history of the Moon (about 3.9 – 4.4×10^9 yr ago) has been found in lunar samples; (5) there are no apparent changes in the character of galactic cosmic rays during at least the past 50×10^6 yr.

Along with this reassuring evidence of constancy, there are some surprising variations, for which the main evidence is the analysis of ancient solar wind particles trapped in lunar samples over the past 2 – 3×10^9 yr. The most puzzling effect is a major variation (about 30%) in the $^{15}\text{N}/^{14}\text{N}$ ratio of nitrogen in the solar wind, from about -200 permil 2 – 4×10^9 yr ago to about $+100$ permil now (R.N. Clayton, University of Chicago; J.F. Kerridge, University of California, Los Angeles), combined with an excess amount of total nitrogen in the ancient lunar soils. Because the Moon contains virtually no indigenous nitrogen, these variations must arise in the solar photosphere, but there is no agreement, and considerable bewilderment, about how such gross isotopic changes can be produced and why they appear in only a single chemical element.

The Conference made possible an exciting introduction, review, and dialogue about the sun's history. What comes next? ●Reworking of current solar theories to include the hard data now available about the ancient and modern Sun. For example, how are such 'proxy' indicators as sunspots and the intensity, energy, and composition of solar wind and solar flare particles related to overall solar luminosity?

●More and better solar measurements, especially of the solar constant (S) and the solar radius. New space missions such as Solar Polar to observe the Sun, and the Venus Orbiter with Imaging Radar (VOIR) to scan ancient planetary surfaces.

●New analytical methods — accelerator measurement of new cosmogenic nuclides such as ^{10}Be , and ion microprobe studies of ancient solar wind implanted in Moon rocks and meteorites.

●Studies of newly available materials that contain important parts of the solar record: micrometeorite spherules from deep-sea sediments; deep cores of polar ice that go back more than 8,000 years; new collections of meteorites that fell in the Antarctic and were preserved in the icecap, some for more than 10^6 yr; old historic records of iron meteorites that may actually record the Maunder Minimum; and a wide variety of historical records, especially from Oriental countries, that have never been examined in detail.

If the problems of deciphering the Sun's history are still immense, so are the new materials and techniques now available to attack them. Scientists from many different fields have many challenging and exciting things to do before they meet again to talk about what the Sun has been up to.

Drug metabolising enzymes

from A.E.M. McLean

THE mammalian liver responds to an influx of chemicals by making more of the enzymes that oxidise and conjugate the majority of foreign chemicals. Induction of enzyme synthesis by carcinogenic hydrocarbons was shown in the rat by Alan Conney (Hoffman La Roche, Nutley) more than 20 years ago and he returned to a Ciba symposium in London recently* to describe induction of drug metabolising enzymes in man by dietary factors. High protein diets, charcoal broiled steaks, and brussel sprouts all have potent inducing effects.

Discussion ranged on the relative importance of natural compared with synthetic inducers such as drugs and polychlorinated biphenyls in the environment. The importance of genetic factors compared with environmental inducers in controlling drug metabolism, was brought up by R. Smith (St. Marys Hospital Medical School, London) who presented information about the different products of metabolism of debrisoquine and phenacetin. He pointed out that the same plasma half life for a drug might be, and often was achieved by quite different genetically determined pathways of metabolism, so that it was important to know not only the rate of disappearance of a drug, but what products were formed.

The need to know the fate of toxic materials was a recurrent theme. Only by measuring the exact fate of carcinogenic compounds like benzo (a) pyrene was it possible to see what influence exposure to inducing compounds, which can increase both toxic and detoxicating pathways, would have on toxicity. Most carcinogens require metabolism to their active form before they will attach DNA, and the sweet potato poison, ipomeanol, similarly needs oxidative activation in the Clara cells of the lung before it causes acute lung damage (M. Boyd, National Cancer Institute, Bethesda). The pathways of activation and detoxification are so numerous that it is difficult to predict whether previous exposure to inducers will increase or

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*The Proceedings will be published as Ciba Foundation Symposium 76 under the title of 'Environmental Chemicals, Enzyme Function and Human Disease' by Excerpta Medica, Amsterdam in Summer, 1980.

decrease the toxic effects of other chemicals.

A. Breckenridge (University of Liverpool) described the effect of inducer drugs in rendering low dose oral contraceptives inactive. It is not clear whether brussel sprouts would have the same effect. A. McLean (University College Hospital Medical School) found no detectable effect on mortality of people with epilepsy of the inducing effects of anticonvulsant drugs. He suggested that the next line of investigation in toxicology should be to examine the response of cells to attack by activated toxic molecules. E. Farber (Toronto) also suggested that too much attention had been paid to the chemistry whereby a molecule of toxic material was activated and interacted with cell macromolecules. Not enough thought had been given to the response of the cell. If 10% of the genome was devoted to response to adverse conditions it would be a reasonable proportion, and leave an enormous range of built-in responses to toxic attack. Carcinogenesis might be the end stage exaggeration of such an adaptive response to a chemically unfavourable environment. A system for development of tumour nodules in a few weeks promised to be a favourable model for analysis of the cell response to carcinogens.

T. Connors (MRC, Carshalton) and J. Higginson (IARC, Lyon) started and ended the meeting by reference to the need to bring human and laboratory experience together, the need to understand tumour promotion as well as carcinogen activation, and the need to combine biochemistry and epidemiology. □

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The ecology of the footprint

from Peter D. Moore

THE ecological effects of recreational pressures on mountains, sand dunes and wetlands are reasonably well documented, and a great deal of work has been carried out and much has been written on the subject of trampling. What is surprising, in view of this, is that so little is known of the ecology of the footprint.

That the footprint has a distinctive ecology of its own was clearly demonstrated by some accidental experiments of Harper, Williams and Sagar some 15 years ago (*J. Ecol.* 53, 273; 1965). They were in the process of examining the germination characteristics of three plantain species (*Plantago lanceolata*, *P. major* and *P. media*) in open plots, when an oversight led to the

trampling of their plots by pigs involved in other experiments. Groups of *Plantago* seedlings appeared wherever a hoofprint had been made. Evidently the alteration of microtopographic, and hence microclimatic and microhydrological features of the soil surface by the impact of the hoof, had strongly influenced the germination and establishment of the plantains. Harper *et al.* developed these observations into a series of experiments in which they confirmed the importance of microtopography in seed and seedling behaviour.

Since then, many attempts have been made to examine the microclimatological changes associated with trampling, both above and below the soil surface. A range of soils, from chalk (Chappel *et al. J. appl. Ecol.* 8, 869; 1971) to sand (Liddle & Grieg-Smith *J. appl. Ecol.* 12, 893; 1975) have been looked at. But in these studies the emphasis had changed from the single footprint to the overall influence of continuous trampling in creating tracks and paths.

One habitat in which the individual footprint survives and remains a scar on the surface for a considerable time is the *Sphagnum* bog. All mire systems are sensitive to trampling for, instead of a mineral soil being compressed, the plant body itself and the organic detritus underlying it, receives the direct impact of the foot. Relatively deep depressions are formed which may fill with water, modifying the microenvironment and creating small scale pool successions. Slater and Agnew (*Biol. Conserv.* 11, 21; 1977) have investigated the development of footprints at Borth Bog in west Wales. They estimated that the 1,890 visitors to the bog each year (mainly researchers and students) displaced a total of 4,228 m³ of the bog surface by trampling. Taking a single footprint, about 50% of the displaced volume was restored in the first month, largely by the elastic response of the bog moss carpet. Subsequent recovery was far slower, being about 90% complete only after 18 months; often it took 30 months for all traces of the footprint to disappear. One species which seemed particularly well adapted to colonisation of disturbed sites was the white-beaked sedge (*Rhynchospora alba*), which, the authors contend, may increase the susceptibility of the vegetation to fire, adding to the ecological complications caused by trampling.

A further example of the distinctive ecology of the footprint has now been reported from the edges of a glacier in northern Norway by Theakstone and Knighton (*Arctic Alpine Res.* 11, 353; 1979). The level of a lake at the outlet of the Austerdalsisen glacier has been lowered 70 m by the construction of a flood control

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tunnel, and the sediments exposed are now being invaded by terrestrial vegetation. The footprints of reindeer and man in the exposed silts have become colonised by the moss *Aongstroemia longipes*. The depressions are 1-2 cm deep and were first created in the summer of 1976. The colonisations observed in 1977 have persisted into 1978, the moss patches still displaying the shapes of the original foot or hoof prints. One suggestion is that the ponding of water in these hollows may have provided the appropriate hydrological conditions for the moss to establish itself. It is interesting to note that the first British report of *Aongstroemia longipes* was in the Ben Lawers area following soil disturbance as a result of dam construction (Crundwell, *Trans. Brit. Bryol. Soc.* 4, 767; 1955).

Evidently the ecology of footprints warrants further study. Such research may confirm the old maxim that one plant's depression may be another's opportunity.

Transformation and tumorigenesis

from R.E. Langman

AT a recent workshop on transformation mechanisms* M. Cohn (Salk Institute) opened with a plea that the meeting address the question of how many genetically determined events are required to convert a cell from the normal to transformed (tumorigenic) phenotype in the absence of any host surveillance mechanisms. In practical terms the change from anchorage-dependence to anchorage-independence (that is, the cells form colonies in agarose) is almost universally accepted as the assay for transformation. But we know virtually nothing about why colony formation in agarose indeed correlates so well with tumorigenicity, except to note that lymphohaemopoietic cells also normally grow as colonies in agarose, and are not obviously transformed or tumorigenic *per se*. In seeking possible generalisations, Cohn argued that there were few ways a cell could become transformed, one linked to anchorage-independence for non-haemopoietic cells, the other linked to whatever causes lymphohaemopoietic transformation.

I. Weissman (Stanford University) showed that individual lymphoma cell lines from mice produce a virus that binds specifically to that lymphoma, and monoclonal antibodies which block virus binding also block cell growth. Thus, it was argued in this case that virus, by perhaps acting like an antigen, provides the proliferative stimulus for the cell it infects. In general then one might view the lympho-

*An Armand Hammer Cancer Workshop was held at the Salk Institute on 12-16 November, 1979.

haemopoietic cells as anchorage-independent by differentiation, but geared to require constant signalling for continued growth, as seen in this viral lymphoma example.

Using the DNA 'transfection' technique, B. Shilo (Massachusetts Institute of Technology) showed that DNA from several cell lines transformed with carcinogens could cause 3T3 fibroblasts to acquire the fully transformed phenotype of anchorage independence and tumorigenicity. The frequency of 'transfection' in these experiments is consistent with the idea that a single dominant gene is responsible for the transformed phenotype; this had been shown previously in the majority of cell hybridisation experiments. Furthermore, transformation of normal recipient cells with the transferred DNA was associated with a unique restriction fragment of DNA. Thus one, or a cluster of tightly linked genes is capable of dominantly causing the expression of the transformed phenotype. The analysis of this 'transforming' fragment and the characterisation of the protein product (if any) will be awaited with interest.

It is well established that the *src* gene of avian sarcoma viruses such as Rous sarcoma virus (RSV) is necessary for expression of the transformed phenotype. As yet it is not quite proven that the *src* gene product alone is sufficient, but the available evidence strongly points in this direction. The 60,000 molecular weight (60K) protein now thought to be coded by the *src* gene is a protein kinase and is very similar to a host cell protein kinase. T. Hunter (Salk Institute) showed that an *in vitro* rabbit reticulocyte protein synthesising system primed with RSV RNA produced a 60K protein that is indistinguishable from the protein identified in RSV-transformed cells. It was also shown that the 60K Src protein is a tyrosine kinase, and that in normal cell extracts phosphotyrosine is a minor component of the phosphorylated amino acids, but that after RSV transformation, the amount of phosphotyrosine, relative to phosphoserine, was increased 5–10 fold. A. Levinson (University of California, San Francisco) reported that the 60K Src protein is tightly bound to, and therefore probably integrated into, the plasma membrane. After mild proteolysis a 56K protein is found free from the membrane, and retains the kinase activity. Attempts to label the 60K Src protein on the outside of the cell have been unsuccessful up to now, and indicate that it is attached to the inside of the plasma membrane. As the Src protein kinase is very similar to the normal cell kinase but is distinct both structurally and in its kinase activity, the suspicion is that the viral Src product initiates transformation by radically upsetting the regulatory functions of the normal cell 'Src' kinase. How this upset of kinase activity leads to transformation is not

Palmdale bulge: fact or fiction?

from Peter J. Smith

SOME curious things have been happening to southern California recently; so much is certain. The only problem is that the curious phenomena that everyone thought were taking place may not have been taking place at all, whereas some quite different curious behaviour may have been going on. Or, what appeared to be certain facts with uncertain explanations now seem to have been uncertain facts with even more uncertain explanations.

Bits of the story have been coming out at intervals for several months now, although the full extent of the problem only became clear at last month's meeting of the American Geophysical Union in San Francisco. The most startling piece of experimental evidence was presented by A.E. Niell of NASA's Jet Propulsion Laboratory, who reported that the distance between his Pasadena laboratory and NASA's Goldstone Deep Space Station some 193 km to the northeast had increased by 20.3 cm in just 7 months. This remarkable measurement was made by repeatedly recording radio noise from quasars simultaneously at the Pasadena and Goldstone radio telescopes, a procedure that can give distance changes between the telescopes to within ± 5 cm; but the expansion has been confirmed in general terms by laser ranging experiments carried out independently by the US Geological Survey. According to J.C. Savage and W.H. Prescott, laser measurements show that between 1974 and 1978 southern California was undergoing a nearly uniform contraction in the north-south direction but that since 1978 there has been east-west expansion with little or no north-south compression. So the laser data for the post-1978 period are consistent with the 1979 radio interferometry.

Unfortunately, however, the laser data as a whole seem not to be in agreement with the behaviour of the so-called Palmdale bulge, the 84,000 km² area of southern California which covers the southwestern section of Niell's interferometry baseline and which has undergone well-publicised ups and downs over the past 20 years. The rise of this zone apparently began in 1959, although this was not discovered until 1976 when it immediately set off speculation about an impending

earthquake. By 1974 the maximum uplift had reached 45 cm; but since then there has been a remarkably rapid deflation accompanied by a tilt of the whole region to the north, an increase in the horizontal strain and an increase in radon emission — a group of phenomena which has again sparked off seismic speculations. The strange thing is, however, that the behaviour of the bulge is inconsistent with much of the laser-derived information about the larger area. When southern California was undergoing north-south contraction from 1974 to 1978, a simultaneous east-west or vertical expansion was to be expected. But during this period the bulge was actually contracting, which it continued to do even when the north-south contraction stopped. As Barry Raleigh of the US Geological Survey recently noted, "no physical model has yet been proposed which incorporates these apparently inconsistent results."

And maybe no model is necessary, for what if the observations are wrong or perhaps, let us say, not quite what they appear to be? After pouring more than \$1 million into the study of a supposedly deflating bulge, this may seem an odd point to make; but D.D. Jackson and W.B. Lee made it at San Francisco in all seriousness. From an examination of the data going right back to 1959, especially those along the profile San Pedro — Los Angeles — Saugus — Palmdale, Jackson and Lee conclude that most, if not all, of the rise centred on Palmdale never in fact took place at all. They find that the altitudes of larger features such as mountains, as indicated by the raw observations, have changed by the same amounts and at the same times as those of the smaller features such as ridges; and although a tectonic cause for such behaviour cannot be ruled out completely, the simplest explanation is the presence of a systematic error, probably in the calibration of the levelling rods.

Many people are simply not going to believe that, at least without much more evidence, if only because of the embarrassment it would cause. Yet there is clearly something going on in southern California. Monitoring continues.

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clear, except that Src protein is required continuously for the expression of the transformed phenotype, as evidenced by temperature-sensitive mutants of *src*.

Although the Abelson leukaemia virus (ALV) lacks an identifiable *src* gene, O.

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Witte (Massachusetts Institute of Technology) showed that a 120K protein with tyrosine kinase activity is produced from the ALV genome. This kinase differs from the RSV kinase in that it requires Mn²⁺ instead of Mg²⁺ cations, but is similar inasmuch as there is in murine bone marrow cells (the preferred viral target in

vivo) a 120K tyrosine kinase with properties very similar to the viral kinase. Although this extends the generality of tyrosine kinase activity as a crucial factor in transformation, it is clear that the small DNA tumour viruses such as SV40 and polyoma lack identifiable kinases. T. Benjamin (Harvard University) noted that mutants in the *hrt* gene region in polyoma virus, which overlaps with the regions encoding the big, middle and small 'T' antigens, showed decreased levels of histone acetylation compared with wild-type polyoma. All these findings on viral transformation together point to several pathways leading to an ultimate transformation site, and to the ability of different viruses to tap into these different pathways to yield the same overall result.

Returning to the opening question of how many genetic events are required for expression of the transformed phenotype, it seems clear that one is a possibility, but that how many different events can give this apparently single gene change is less clear although one must suspect that it is greater than one. Epidemiologists have argued for multistage models of *in situ* tumorigenesis, especially for carcinomas where a minimum of between four and six steps is predicted. If one (dominant) or two (recessive) gene level events can give rise to *in vitro* transformation, then the suspicion must be that the remainder of these steps are either spurious because of assumptions about the data and its relation to tumorigenesis, or are due to evasive steps a transformed cell must take in order to survive various surveillance mechanisms. □

Polarised electrons are going round in circles

from C. B. Lucas

SPIN-polarised electrons are of interest to physicists and biologists at both the high and low ends of the particle energy spectrum. Several recent advances have provided new sources of polarised electrons, and should provide new insights in fields such as neutral currents, surface physics and molecular optical activity.

One way of producing spin-polarised electrons is to take a storage ring, fill it with electrons or positrons, and leave for an hour or so. This recipe for polarising electrons by macroscopic means seems contradictory to long-standing arguments that they cannot be so polarised, for example by passage through a Stern-Gerlach magnet. In that case, the splitting of the beam after passing through an inhomogeneous magnetic field is less than the uncertainty in their position as given by Heisenberg's principle.

However the polarisation mechanism is easily understood if one remembers that an electron orbiting in the magnetic field of a storage ring and emitting synchrotron radiation may flip its spin on emitting a photon. The probability of a transition depends on the spin direction relative to the guiding magnetic field, the positrons becoming polarised in the direction of the field, the electrons in the opposite direction. Since the probability of a spin-flip emission is typically only 10^{-11} that of a normal emission, the polarisation builds up very slowly.

The polarisation effect was first predicted by Ternov, Loskutov and Korovina as early as 1961. Two years later it was shown that, in a typical storage ring, the transverse polarisation should build up in a period of about an hour to a limiting value of 93.4%.

The first experimental confirmations of theoretical predictions were made on electrons in the storage rings at Novosibirsk and Orsay in 1971. In the former, at an energy of 640 MeV, approximately 50% polarisation was measured after about 100 minutes, compared with a calculated value of 66% when depolarisation effects were included. Since then, measurements on several of the world's storage rings have been made, confirming these results, and $76 \pm 5\%$ polarisation has been reported from Stanford. (The latest position was discussed at the Argonne conference on High Energy Physics with Polarised Beams and Polarised Targets (*AIP Conference Proceedings* No 51, ed. G.H. Thomas, 1979)).

Another example of a circular approach to this problem is the latest experiment to produce polarised electrons by photoionisation with circularly polarised light. This process is known as the Fano effect, after its originator. In this case, polarised electrons are produced by transfer of angular momentum from the photons to the electrons. The first Fano-effect studies were made in the near ultraviolet with caesium, and were remarkable in producing electrons, almost completely polarised, with wavelengths close to a minimum in the photoionisation cross section.

Measurements in the vacuum ultraviolet require more sophisticated optical techniques. Measured polarisation at wavelengths longer than 140 nm ranges from a maximum of about 50% in thallium to, typically, a mere 15% in lead. Molecules have been even more disappointing, yielding no significant polarisation of the photoelectrons.

Nevertheless measurements have been continued further into the vacuum ultraviolet, at around 100 nm, by a group using the radiation produced by the synchrotron running at 800 MeV at the University of Bonn (*J. Phys. B* 12, L679; 1979). Preliminary measurements showed that the radiation above and below the

plane of the electrons was respectively left and right circularly polarised to at least 80%. When used to ionise xenon, circularly polarised photons produced electrons which were polarised up to 75%, in general agreement with theoretical predictions. Though still not so impressive as the very first measurements in caesium, these results are comparable with the polarisation which could have been obtained if the electrons in the synchrotron had been injected into a storage ring, though the current would then have been many orders of magnitude greater. Hence polarised electrons in a storage ring could be used to produce polarised photoelectrons through the synchrotron radiation! This means that the particle physicist is rapidly losing his interest in the polarised electrons produced by the atomic physicist, since the former has a very intense source of highly polarised energetic electrons (and positrons) accidentally at his disposal; this provides the opportunity to study directly the neutral weak current phenomenon.

The atomic physicist can unfortunately make little use of polarised electrons or positrons at such high energies, and the laws of electron optics prevent a useful well-defined low energy beam from being obtained from one of much higher energy. Of interest, therefore, is the report by a group at the University of Michigan (*Phys. Rev. Lett.* 43, 1281; 1979) that 1 eV positrons have been produced with at least 20% polarisation. The authors suggest that the principal use of this source to the physicist will be for the study of polarised low-energy positron diffraction from surfaces. For the biologist, interest in this source is connected with the controversial hypothesis that the origin of biomolecular optical activity might be linked to the polarisation of β -particles from radionuclides.

Interest in spin-polarised electrons is burgeoning as the number of different mechanisms for their production has increased rapidly in the past decade; previously, only elastic electron scattering by heavy atoms looked at all useful. Production of polarised electrons by the use of lasers has not yet proved to be particularly important. Developments in multiphoton ionisation of alkali atoms look very promising, since the atom can be pumped into a fully polarised excited state and then photoionised with a second circularly polarised laser beam. The electrons are then not only expected to be almost fully polarised, but also to have the least energy spread of any source of electrons, so that interesting experiments with low energy polarised monochromatic electrons should become possible. □

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REVIEW ARTICLE

Intermediate filaments as mechanical integrators of cellular space

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Five chemically distinct classes of intermediate filaments can be identified within higher eukaryotic cells. Each class is characteristic of a particular cell type. These filaments may function to integrate mechanically the various structures of the cytoplasmic space in a way that is tailored to the differentiated state of the cell.

FOR some years electron microscopy has identified a major filamentous system in the cytoplasm of higher eukaryotic cells that is distinct from actin filaments, microtubules and myosin filaments. As their characteristic diameter of 100 Å is between that of the smaller actin filaments (60 Å) and the larger microtubules (250 Å) and myosin filaments (150 Å), this new filamentous system has come to be known as the 'intermediate filaments'. Intermediate filaments were at first widely regarded as disaggregation products of either microtubules or myosin filaments and as a result they were neglected. But new biochemical and immunofluorescent techniques have recently established intermediate filaments as a distinct fibrous system composed of chemically heterogeneous subunits. The subunit structure defines five major classes of intermediate filaments that can be distinguished both biochemically and immunologically: (1) keratin (tono) filaments, found in epithelial cells and cells of epithelial origin; (2) desmin filaments, found predominantly in smooth, skeletal and cardiac muscle cells; (3) vimentin filaments, found in mesenchymal cells and cells of mesenchymal origin; (4) neurofilaments, found in neurones; and (5) glial filaments, found in all types of glial cells. It is common to find two of these classes co-existing in the same cell. This review highlights the most recent studies which have led to these conclusions and focuses on certain observations that shed light on the cytoplasmic function of these filaments. From these studies, it seems that intermediate filaments have a very important role in the mechanical integration of various cytoplasmic organelles.

Keratin filaments

Keratin filaments are perhaps the most atypical of all intermediate filament classes. Mammalian keratins are a group of intracellular α -fibrous proteins associated in a filamentous form (tonofilaments) with an approximate diameter of 80 Å. They are actively synthesised by the inner, living cell layers of the mammalian epidermis and represent its major differentiation product. Eventually, the keratins form the bulk of the outer, fully differentiated, dead, stratum corneum layer¹.

In addition to being the major differentiation product of epidermal cells, keratins are present in certain other stratified squamous epithelia^{2,3}, but they are absent from cells of mesenchymal origin (Table 1). Antibodies to the mammalian keratins have been used in immunofluorescent studies to identify many epithelial cells in culture or in tissue sections⁴⁻⁷. Generally, these antibodies show a wide cross-reactivity among species, indicating that different keratins are immunologically related. In cultured epithelial cells, the keratin filament bundles which react with the antibodies form an elaborate array throughout the cytoplasmic space (Fig. 1). Electron microscopy shows that bundles of keratin tonofilaments often terminate in membrane-bound desmosomes⁴⁻⁷.

Keratins are insoluble in neutral aqueous buffers, but can be extracted in the form of a multichain unit^{8,9} at low pH or by denaturing solvents (for example, 8 M urea). A reducing agent is also required for the efficient solubilisation of keratins from the terminally differentiated cells. Electrophoresis in denaturing gels separates keratin into a family of polypeptides with molecular weights (MW) ranging from 40,000 to 65,000 (refs 10-14 and refs therein). Keratins extracted in the presence of urea from the living cells of epidermis¹¹⁻¹³ or from cultured human keratinocytes¹⁴ are composed of six to seven major polypeptides which have MWs of 47,000 to 58,000. Dialysis of the urea extracts against a dilute salt solution results in the spontaneous formation of filaments without any obvious requirement for a specific cofactor(s) such as divalent cations and nucleoside triphosphates. The reconstituted filaments show the same polypeptide composition, morphology, characteristic diameter of 80 Å and X-ray diffraction patterns as native filaments¹⁰⁻¹⁴. Extraction and reconstitution experiments suggest that most keratins of living cells exist in a reduced form that favours polymerisation and become increasingly cross-linked by intermolecular disulphide bonds as differentiation proceeds¹²⁻¹⁴.

Most combinations of two or three purified keratin polypeptides polymerise *in vitro* into filaments of the same general structure as native keratin filaments. With two polypeptides, reconstitution is in the precise molar ratio of 1:2 whereas with three polypeptides equimolar reconstitution is the rule¹². These ratios are maintained even in filaments polymerised from mixtures containing disproportionate amounts of polypeptides, indicative of a highly specific interaction between the various keratin polypeptides¹². X-ray diffraction data had also indicated that native epidermal keratin filaments are composed of a fundamental three-chained structural unit¹⁵⁻¹⁷. As the keratins are composed of six or seven polypeptides, many triple-chained units of similar structure but of different polypeptide composition must be possible. Thus, several different triple-chained units may assemble to form a single filament¹². If this is the case, then both the reconstituted and native filaments are likely to be heterogeneous with respect to polypeptide composition. Such filament heterogeneity may confer specificity to the cytoplasmic function of keratin filaments.

The biochemical relationship between individual keratins has been investigated by several groups^{10-14,18}. Although the keratins of human epidermal cells grown in tissue culture are similar to those of stratum corneum of human epidermal callus with respect to their solubility properties, immunological reactivity and ability to assemble into 80-Å filaments *in vitro*, there are differences in the MWs of some of the proteins and the number of components^{14,18}. In particular, the largest protein of stratum corneum (MW 63,000) is absent from the keratins of cultured human or mouse keratinocytes¹⁴. This high MW keratin seems to be characteristic of the stratum corneum. In another study, the keratins from two epidermal tissues of the same species,

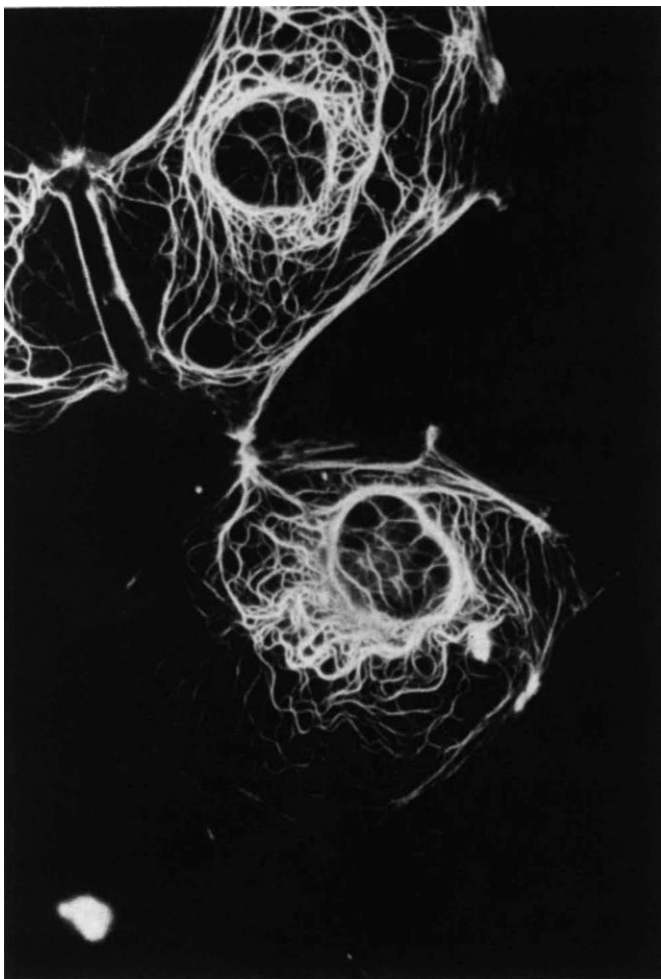


Fig. 1 The distribution of keratin filaments in epithelial cells grown in tissue culture (PtK₂) using indirect immunofluorescence with antibodies to keratin (courtesy of M. Osborn, K. Weber and W. W. Franke¹¹⁰).

bovine hoof and snout, were shown to be similar with respect to their MWs, amino acid compositions and percentage of helical structure¹⁹; however, one of the high MW (67,000) polypeptides from snout was absent from hoof keratin. In addition, the tryptic and cyanogen bromide peptides of total keratins from hoof and snout were distinctly different¹⁹. Thus, not only do differences exist in the keratins between different stages of epidermal cell differentiation, but also in keratins of different epidermal tissues of the same species. Furthermore, all of the keratins found in cellular extracts of cultured human epidermal cells are synthesised *in vitro* by distinct mRNA species isolated from these cells²⁰. The kinetics of translation of these proteins is inconsistent with any type of precursor-product relationship within the keratin family or with the existence of precursors larger than the keratins²⁰. These experiments have also clearly shown that epidermal keratins are a family of closely related but distinct proteins that are evolutionarily conserved.

Similar evidence for the existence of multiple genes was obtained first for the avian feather keratins^{21,22}, a family of proteins with MWs near 10,000 which assemble into 30-Å (tono) filaments and are thus quite different from epidermal keratins. By electrophoretic mobilities, amino acid composition and immunological criteria, the keratins of chicken feathers are distinct from those of scales. Differences exist as well between embryonic and adult tissues²¹. Nucleic acid hybridisation experiments have indicated that the number of genes for these low MW keratins may be 100 to 240 (ref. 22). All these results suggest that distinct sets of keratin genes are expressed during

epithelial cell differentiation, in different developmental stages and in different epithelial tissues.

Desmin filaments and their role in the mechanical integration of myofibrils in muscle cells

It has been suspected that in muscle cells there is a cytoskeleton which holds the constituent myofibrils in place. The presence of structural elements transverse to a muscle fibre's long axis is suggested by the tendency for the Z disks, M lines and other sarcomeric units of neighbouring myofibrils to be in axial register across the fibre, thereby giving rise to the striking cross-striated appearance of the muscle cell²³. Since the early days of light and electron microscopy of muscle, fibrous elements were observed to link myofibrils laterally at their Z, N and M lines and to link them to the surface membrane of the whole fibre²⁴⁻²⁷, but only recently have they been characterised as intermediate filaments. The distribution of the filaments is characteristic of skeletal, cardiac or smooth muscle cells.

While intermediate filaments are present in both the embryonic and adult forms of all three muscle types²⁸⁻³⁵, they are most abundant in adult smooth muscle. Here, these filaments form an interconnected network that links cytoplasmic dense bodies with membrane-bound dense plaques^{30,34,35}. Morphological studies have shown that actin filaments are also inserted into these electron-dense structures²⁹, which can be shown to contain α -actinin and tropomyosin using immunocytochemical techniques³⁶. Thus, smooth muscle dense bodies appear analogous to striated muscle Z lines.

Extractions of smooth muscle cells with high salt buffers renders contractile actin and myosin filaments soluble, leaving behind a cytoskeleton composed predominantly of intermediate filaments still attached to dense bodies^{34,35}. Electrophoretic analysis indicates that this cytoskeleton consists of two major proteins: actin; and a 50,000–55,000-MW protein which we have named desmin (from the Greek noun *δεσμός* = link, bond)^{37,38}. An identical protein has been isolated by Small and Sobieszek from mammalian smooth muscle and has been referred to as skeletin³⁹. On dialysis or neutralisation, purified desmin polymerises into a gel-like network of filaments. Reconstituted desmin filaments have the same average diameter (100 Å) as smooth muscle intermediate filaments^{38,39}. A small amount of actin appears to be stably associated with desmin^{38,40}. The actin bound to desmin is likely to be in a conformation different from thin filament actin. It is not yet known if the interaction of desmin with actin is restricted *in vivo* in its extent and spatial location, but such an interaction might well occur at cytoplasmic or membrane-bound dense bodies, where actin and desmin filaments exist in very close proximity.

The small size of smooth muscle cells and their complicated morphology make *in situ* studies difficult, and for this reason we have used skeletal muscle for our cytological investigations of desmin. Although common in embryonic striated muscle, intermediate filaments become progressively less visible as the sarcomeres condense and become laterally registered. Intermediate filaments are rarely seen in adult muscle⁴¹, but substantial amounts of desmin can be detected in adult striated muscle by electrophoretic and immunological methods^{37,42-44}. Using immunofluorescence on longitudinal sections of myofibrils, desmin was shown to be localised in both skeletal and cardiac muscle Z lines and in regions of cell-cell juncture and apposition of the Z line with the plasma membrane (cardiac intercalated disks)^{37,42,44,45}. Desmin is thus the third protein, in addition to α -actinin and actin, to be localised at the Z line using immunocytochemical techniques⁴⁵⁻⁴⁷. To obtain information on the spatial relationships of these three proteins within the plane of a single Z disk, it was necessary to develop a method which allowed a face-on view of Z disks by light microscopy as well as by electron microscopy. Normally when a muscle fibre is sheared, it is cleaved lengthwise, between laterally associated Z disks, to produce myofibrils, but we discovered that if most of

the myosin and actin are first extracted from the fibre with high salt, the fibre's longitudinal strength is reduced and subsequent blending shears the fibre transversely between Z planes, producing honeycomb-like sheets of Z disks⁴⁸. The generation of sheets of interconnected Z disks lends support to the idea that the Z disks within a Z plane are actually connected to one another. Electrophoretic analysis indicates that these Z planes are composed predominantly of actin and desmin (as is the case with smooth muscle dense body cytoskeletons).

Indirect immunofluorescence performed on a single Z disk sheet reveals that desmin is present at the periphery of each Z disk, forming an interconnecting network across the fibre (Fig. 2a). α -Actinin is localised within each disk, giving a face-on fluorescence pattern that is complementary to that of desmin (Fig. 2b). Actin is present throughout the Z plane, apparently co-existing with both desmin and α -actinin⁴⁸. Thus, the Z disk is composed of two molecular domains, a peripheral one which contains at least desmin and actin, and a central one which contains at least α -actinin and actin. Do these two domains assemble at different times during myogenesis? In the early stages of myogenesis, electron microscopy^{31,32,41} and

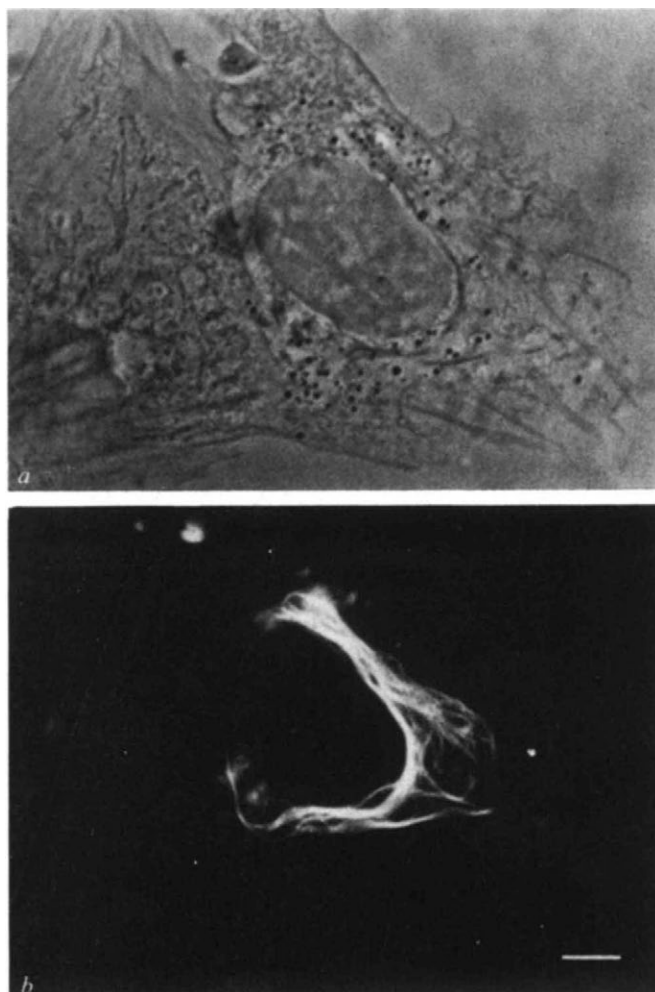


Fig. 3 The perinuclear aggregation of intermediate filaments in primary chick embryonic cardiac cells exposed to colcemid. *a*, Phase contrast image; *b*, fluorescence image. Indirect immunofluorescence using antibodies to chicken smooth muscle desmin. Similar images are obtained using antibodies to vimentin (see text). Scale bar, 10 μ m.

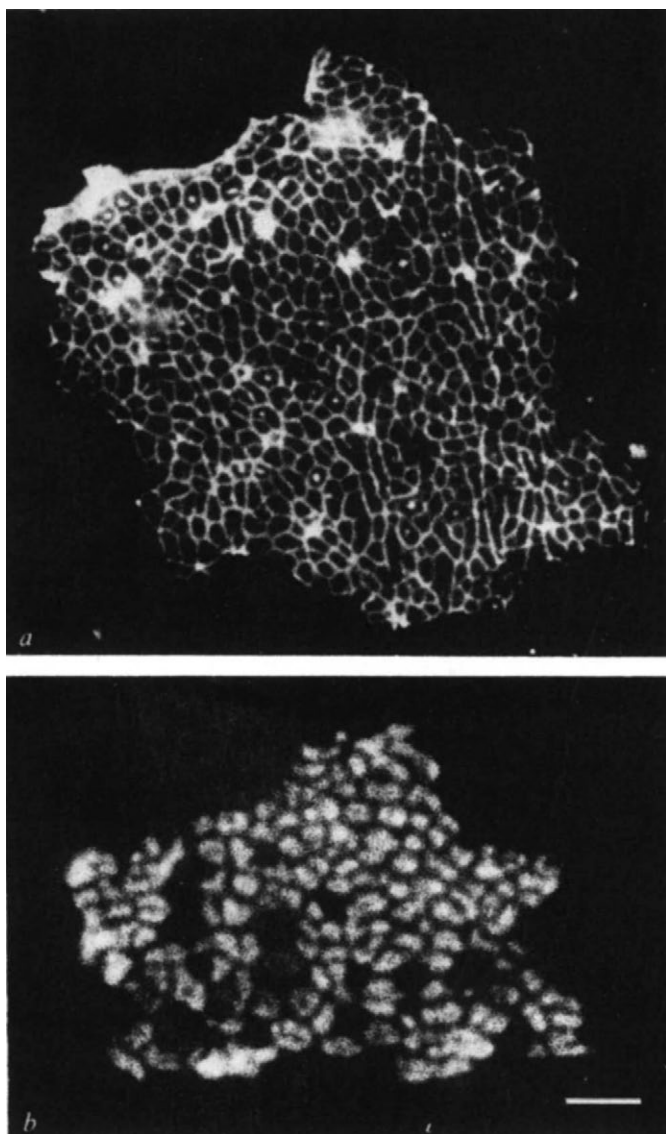


Fig. 2 *a*, The distribution of desmin at the periphery of Z disks in isolated Z disk sheets from chicken skeletal muscle. Indirect immunofluorescence using antibodies to chicken smooth muscle desmin. Vimentin shows the same distribution as desmin in these sheets (see ref. 49). *b*, The distribution of α -actinin in the interior of Z disks in isolated Z disk sheets from chicken skeletal muscle. Indirect immunofluorescence using antibodies to chicken muscle α -actinin. Scale bar, 2.5 μ m.

immunofluorescence with desmin-specific antibodies⁴⁹ reveal that numerous desmin filaments exist scattered in the cytoplasm, not associated with the newly forming Z disks and sarcomeres. During this time the filaments can be induced to aggregate into bundles by exposing the cells to colcemid (Fig. 3)^{31,42,49,50}. Later in development, desmin and intermediate filaments begin to associate with and encircle the Z disks⁴⁹ and become resistant to aggregation by colcemid⁴⁹. Therefore, desmin and other Z disk components may undergo a 'molecular maturation' during myogenesis which allows their association.

In most types of skeletal muscle, the Z disks are encircled by the electrically excitable transverse-tubular (T) membrane. This membranous system is an extension of the plasma membrane and forms junctions (triads) with the terminal cisternae of the calcium-sequestering sarcoplasmic reticulum (SR) membrane which surrounds the rest of the myofibril⁵¹⁻⁵⁶. Electron microscopy of isolated Z disk sheets (Z planes) shows membranous elements, presumably of T-system and SR origin, surrounding the individual Z disks⁴⁸. Those same regions react strongly when the sheets are exposed to specific probes that fluoresce only in a hydrophobic environment⁵⁷. The distribution of the membranous material in Z planes is coincident with that of desmin and complementary to that of α -actinin⁵⁷.

Linkages between adjacent myofibrils may not be restricted to the Z disk. Electron microscopy of actomyosin-depleted myofibrils demonstrates that fibrous linkages exist between adjacent myofibrils also at the M and N lines⁴⁸. In some types of muscle the triadic junctions of the T-system are positioned at the

sites of overlap of the thin and thick filaments (A-I junction)⁵⁶; similar fibrous linkages may be involved in anchoring the triads to the periphery of the myofibril at these sites. The molecular composition of these links is presently unknown.

One seemingly puzzling observation is that in adult skeletal muscle fibres intermediate filaments are only rarely seen by conventional electron microscopic techniques. However, in certain cases, bundles of intermediate filaments have been observed surrounding skeletal muscle Z disks and extending laterally between them^{41,56,58}. The presence of high concentrations of membranous material in the intermyofibrillar space may frequently obscure the presence of intermediate filament connections between adjacent Z disks, M lines and N lines⁵⁹. Similarly, in cardiac muscle, bundles of intermediate filaments appear to surround Z disks and to connect them to the triad junctions irrespective of the position of the triad within the sarcomere (that is, at the Z line or A-I junction)⁶⁰⁻⁶⁴. Occasionally these filaments extend from the Z disks to the nuclear and plasma membranes⁶¹⁻⁶³. Intermediate filaments are especially prominent in hypertrophied myocardial cells⁶¹ or in cardiac muscle cells of animals treated with anabolic steroid hormones⁶⁴.

On the basis of these observations, we have proposed the following functions of desmin and desmin filaments in muscle cells^{37,40,48,65}, a schematic representation of which is shown in Fig. 4. First, desmin may mechanically integrate all contractile actions of a muscle fibre by linking individual myofibrils laterally at their Z disks and by linking Z disks to the plasma membrane and to other cytoplasmic membranous organelles. This arrangement will also ensure the maintenance of the proper

alignment of cellular structures during the contraction-relaxation cycle of muscle. During the biogenesis and assembly of myofibrils, desmin may have an important role in the generation of the striated appearance of muscle by bringing the Z disks into lateral register. Second, desmin may function in the biogenesis of the T-SR membrane system. Some mechanism is required to direct the deposition of this membrane system to the periphery of the Z disk. A protein with both membrane and Z disk affinities, like desmin, could be vital in this process. As desmin binds to actin, desmin may also mediate the attachment of actin at additional membrane sites such as cardiac intercalated disks and smooth muscle dense bodies. We can further hypothesise that this transverse mechanical integrating system of desmin filaments may be an essential feature of all types of muscle regardless of their position in the evolutionary scale⁴⁴. The proposed function of desmin indeed seems to justify its name and provides the first well documented cytoskeletal function for intermediate filaments.

Vimentin filaments

A variety of cells of mesenchymal or non-mesenchymal origin contain vimentin filaments (see Table 1), so called because immunofluorescence studies with antibodies to the major subunit of these filaments reveal a wavy (Latin *vimentus*) cytoplasmic filamentous system (Fig. 5)^{6,73}.

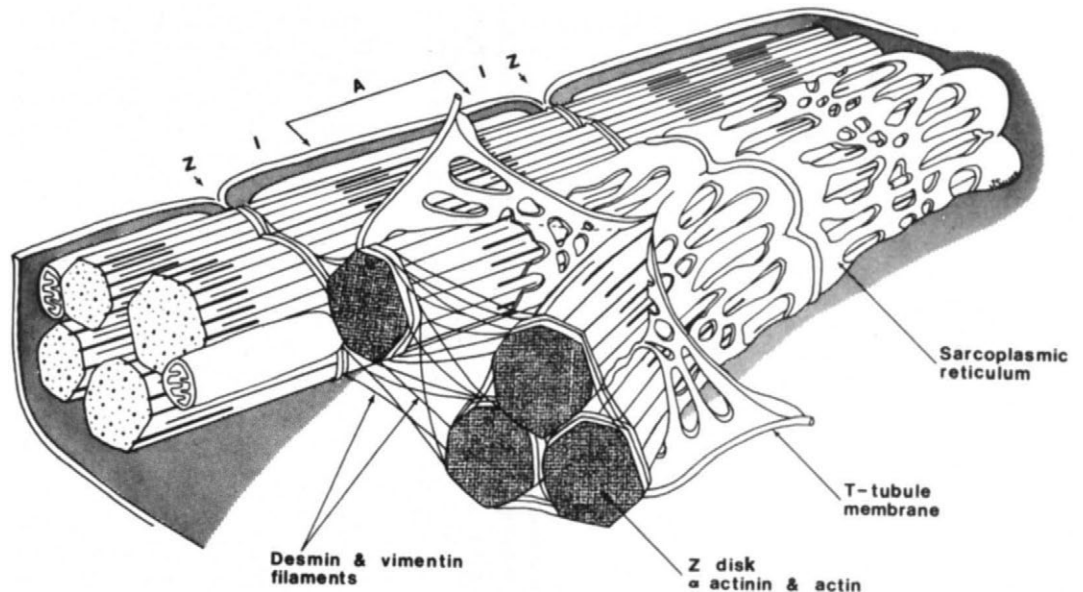
Vimentin was first characterised as a 52,000-MW, major subunit of intermediate filaments in chick embryo fibroblasts⁶⁶. The wide cross-reactivity of vimentin antibodies with cells of mammalian, avian or amphibian origin indicates that this protein is conserved in evolution^{6,67-69}.

Table 1 Presence of intermediate filament subunits in different cell types

Cell type	Vimentin (MW 52,000)	Desmin (MW 50,000)	Keratins (MW 40,000-65,000)	Glial filaments (GFA) (MW 51,000)	Neurofilaments (MW 200,000, 150,000, 68,000)
Adult muscle:					
Skeletal, cardiac, smooth	+	+	-	-	-
Myotubes	+	+	-	-	-
Myoblasts	+	+	-	-	-
Fibroblasts	+	+	-	-	-
3T3	+	+	-	-	-
SV40-3T3	+	ND	-	-	-
BHK-21	+	+	-	-	-
NIL-8	+	-	-	-	-
Sarcoma 180	+	ND	-	-	-
Schwann cells	+	+	-	-	-
Neuroblastoma	+	ND	-	-	ND
Pigment cells	+	-	-	-	-
Skin melanocytes	+	ND	-	-	-
Chondroblasts	+	-	-	-	-
Endothelial cells	+	-	-	-	-
PNS, CNS neurones	-	-	-	-	+
Glial cells:					
Astrocytes					
Oligodendrocytes					
Microglia	+	-	-	+	-
Ependymal cells					
Glioma C6					
Epithelial cells:					
HeLa	+	-	+	-	-
Rat kangaroo PtK ₁ , PtK ₂	+	-	+	-	-
Epidermal cells	-	-	+	-	-
Cultured keratinocytes	+	-	+	-	-
Stratified squamous epithelia	-	-	+	-	-
Epidermal appendages	-	-	+	-	-
Hassal's corpuscles	-	-	+	-	-
of the thymus					
All other epithelial cells	+	-	+	-	-
Exceptions:					
Epithelia of iris and lens	ND	-	-	-	-
Glomerular and tubular cells of kidney	ND	-	-	-	-
Liver hepatocytes	+	ND	+	-	-
Hepatoma cells	+	ND	-	-	-
Pancreas	ND	ND	-	-	-

Compiled from data in refs 3, 4, 5-7, 37, 38, 42-44, 67-69, 73, 86, 95-102, 108, 109. ND, not determined.

Fig. 4 Schematic representation of the distribution of desmin, vimentin, α -actinin, actin and membranous organelles in relationship to chicken skeletal myofibril Z disks.



One of the most interesting and characteristic properties of vimentin-containing intermediate filaments is their aggregation into perinuclear filamentous bundles in the form of a cap or a ring in cells exposed to colcemid⁷⁰⁻⁷³, a finding which originally prompted the erroneous belief that these filaments were degradation products of microtubules. Similar structures exist transiently during normal cell adhesion and spreading onto a substrate^{74,75} or in non-motile tumour cells⁷⁶. Isolated filament caps contain two predominant polypeptides in approximately equimolar amounts, one with a MW of 52,000–55,000 which corresponds to vimentin in other cell types, and the other with a MW of 50,000–54,000 which, as discussed below, corresponds to muscle desmin. Vimentin and desmin can be purified from the birefringent caps by repeated cycles of disassembly–reassembly, induced by lowering and raising the ionic strength of the extracting solutions^{75,77}. Reassembled filaments have an average diameter of 100 Å; it is not known whether vimentin and desmin copolymerise into a single filament or whether they polymerise into distinct filaments with common properties. Vimentin has now been identified in a variety of cell types, quite frequently in co-existence with the major subunit of some of the other classes of intermediate filaments (see below).

Vimentin filaments are very insoluble, suggesting that they have a structural function in the cytoplasm. They frequently terminate at both the nuclear membrane and the adhesion plaques of detergent-extracted cytoskeletons prepared from cultured fibroblasts^{78,79} or nucleated chicken red blood cells⁸⁰. In normal conditions (in the absence of colcemid), there is always a certain perinuclear concentration of 100-Å filaments in the cell lines studied⁷⁹. Enucleation of cells by cytochalasin B produces karyoplasts surrounded by intermediate filaments and cytoplasts in which intermediate filaments remain situated in the central region of the cytoplasm⁷⁹. Together these results strongly suggest that vimentin is tightly associated with the cell nucleus, possibly providing it with mechanical support and constraining it in a specific place in the cell⁷⁸⁻⁸⁰. In this respect, several other observations have also been made of the association of intermediate filaments with centrioles *in situ*^{75,81}.

The fate of vimentin filaments during the cell cycle appears to vary from one cell type to another. In BHK-21 cells the filaments disaggregate during prometaphase and reappear as filamentous caps in the perinuclear area during the spreading of the two daughter cells⁷⁵. However, in most other cell types (NIL-8, endothelial cells), the filaments appear to persist in a polymerised form throughout mitosis^{72,73,82}. In vascular endothelial cells, the interphase cells contain a ring of intermediate filaments that encircles the nucleus and is maintained in a plane parallel to the substrate^{72,82}. During prophase and metaphase

the cells round up and the filament ring becomes wavy, although it still exists as a closed structure. During anaphase the ring elongates into a rectangle that contains the spindle apparatus and chromosomes. In late telophase, cytokinesis cleaves the filaments into crescents at the site of the contractile ring, which then close into rings in the daughter cells⁸² (see Fig. 6).

Neurofilaments and glial filaments

Neurofilaments (average diameter 100 Å) and microtubules (250 Å diameter) comprise the main structural elements of neuronal axons, dendrites and perikarya. The neurofilaments can be either randomly or uniformly dispersed and appear to be linked to each other through wispy sidearm appendages. Glial filaments are found randomly dispersed in the cytoplasm of astrocytes and other types of glial cells; in contrast to the neurofilaments, glial filaments are more closely packed (average diameter 80 Å), are less parallel in their orientation and lack sidearm appendages⁸³.

There has been prolonged controversy and confusion over the subunit composition of neurofilaments and glial filaments. The first intact intermediate filaments to be isolated⁸⁴ were derived from myelinated nerve fibres and were therefore assumed to be characteristic neurofilaments with a 51,000-MW protein derived therefrom being the neurofilament protein. This assumption was erroneous. It has since been clearly demonstrated that these filaments were predominantly glial filaments with a 51,000-MW glial fibrillary acidic (GFA) protein as their major subunit.

When neurofilaments are purified from the peripheral nervous system (PNS), which is relatively free of glial cells, no protein with a MW of 51,000 is detected^{85,86}. Instead, rat peripheral nerve, rat spinal cord and rabbit intradural spinal nerve roots⁸⁵⁻⁸⁸ each contain three major polypeptides with MWs of 200,000, 150,000 and 68,000. In contrast, squid neurofilaments are composed of only two major polypeptides with MWs of 200,000 and 60,000 (refs 89–91), while those from the giant axon of the marine worm, *Myxicola infundibulus*, are composed of two polypeptides with MWs of 160,000 and 150,000 (refs 89, 92). In spite of these differences, however, one of the characteristic properties of both vertebrate and invertebrate neurofilaments is their extreme susceptibility to proteolysis in the presence of Ca^{2+} ions^{92,93}.

The biochemical evidence distinguishing neurofilaments from glial filaments has been supported by immunological studies with antibodies prepared with and specific for the purified subunit proteins of neurofilaments and glial filaments. Antibodies raised to the 68,000-MW components of mammalian peripheral nerve react in immunofluorescence with axons and

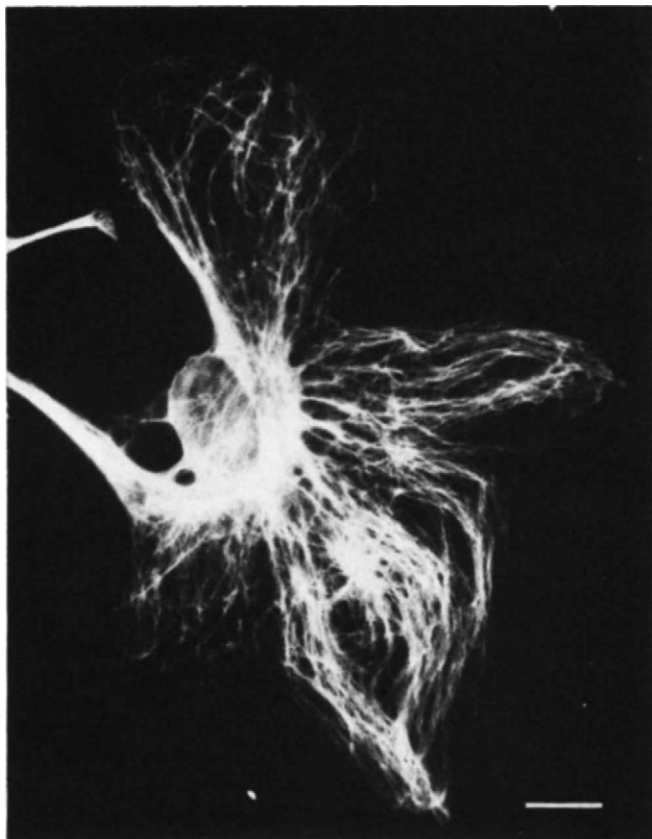


Fig. 5 The distribution of intermediate filaments in a normal chick embryonic fibroblast using antibodies to chicken vimentin in indirect immunofluorescence (courtesy of B. L. Granger). Scale bar, 10 μ m.

neurones throughout mammalian PNS and central nervous system (CNS) but do not react with glial cells^{86,94,95}. Similarly, antibodies to GFA react in immunofluorescence only with glial cells or cells of glial origin and do not react with neurones or their processes^{86,96,97} (for a review on GFA and its properties see ref. 98). Antibodies to GFA do not react with muscle cells, myofibrils, or fibroblasts, indicating it is immunologically distinguishable from vimentin and desmin⁴⁴. Conclusive demonstration that GFA is the major component of glial filaments stems from the observations that (1) GFA is the major component of purified glial filaments⁹⁹; (2) purified GFA will assemble *in vitro* into filaments with an average diameter of 100 Å (ref. 98); and (3) antibodies to GFA will react with glial 100-Å filaments *in situ* using the immunoperoxidase method⁹⁶.

While the function of glial filaments is presently undetermined, neurofilaments appear to function as a three-dimensional structural lattice that provides tensile strength to the axons. This idea stems from the observation that the extruded axoplasm from *Myxicola* is a highly structured gel consisting almost exclusively of neurofilaments^{91,92}. Exposure of this gel to Ca^{2+} results in the degradation of neurofilaments and the dissolution of the gel.

Co-existence of different intermediate filament subunits in the same cell

A number of cells have recently been shown to possess the subunits of at least two distinct intermediate filament types, sometimes in clearly distinguishable filamentous systems. For example, PtK₂ and HeLa cells have abundant keratin filaments in their cytoplasm and contain vimentin in the perinuclear aggregates of intermediate filaments experimentally induced by colcemid^{6,7}. Both polypeptides can also be demonstrated in these cells by electrophoretic analysis of their filamentous detergent insoluble cytoskeletons^{6,7}.

Similarly, two-dimensional electrophoresis of the cytoskeletons from fibroblast-free cultures of chicken embryonic muscle, chicken embryo fibroblasts, 3T3 cells and BHK-21 cells shows that all these contain not only vimentin but also a second polypeptide which is highly homologous to muscle desmin^{100,101} and can be detected by immunofluorescence in colcemid-induced perinuclear rings of intermediate filaments¹⁰⁰. In skeletal myotubes and isolated myofibrils, the distribution of desmin and vimentin coincides⁴⁹.

Recent immunofluorescent experiments also suggest that glial cells (glioma cells and astrocytes) contain vimentin in addition to the glial-specific filament subunit (GFA)^{6,68,69,102}. In cultured astrocytes, vimentin and glial filaments can be distinguished by their characteristic immunofluorescence patterns. The possible presence of vimentin in purified glial filaments and the natural occurrence of autoantibodies to vimentin may explain the wide cross-reactivity observed in earlier studies with antibodies to GFA¹⁰³.

These observations demonstrate that desmin and vimentin can co-exist in muscle and non-muscle cells; similarly, vimentin and certain keratins can co-exist in some epithelial cell types, and vimentin and GFA can co-exist in glial cells. While vimentin and keratin filaments can be distinguished easily by electron microscopy and their responses to colcemid, this is not the case with vimentin and desmin filaments. Both desmin and vimentin filaments appear morphologically (that is, by transmission electron microscopy) very similar and aggregate into perinuclear rings in cells exposed to colcemid (see Fig. 2b). As desmin and vimentin from BHK-21 cells co-purify through cycles of polymerisation-depolymerisation (see above), it will be intriguing to find out whether the two polypeptides can co-polymerise into one and the same filament, as is the case with the keratins, or whether they polymerise into distinct filaments. Immunoelectron microscopy with desmin and vimentin antibodies should answer these questions. At present, the function of desmin in non-muscle cells and of vimentin in muscle cells is unknown, but by analogy to muscle cells, desmin may be involved in the adhesion of actin filaments to the plasma membrane.

Phosphorylation of intermediate filament subunits

All intermediate filament subunits examined to date seem to be phosphorylated *in vivo* and can be phosphorylated *in vitro*. For example, phosphorylation of the 200,000 but not the 60,000-MW polypeptide from squid axoplasm occurs after intracellular injection of [γ -³²P]ATP into giant axons or addition of [γ -³²P]ATP to extruded axoplasm⁹⁰. In contrast, both *Myxicola* neurofilament polypeptides are phosphorylated *in vivo*, probably by a soluble kinase which is independent of Ca^{2+} cyclic AMP or cyclic GMP for its activity¹⁰⁴. A portion of this activity remains associated with neurofilaments even after extensive purification of the filaments¹⁰⁴. Similarly, all three polypeptides of the mammalian neurofilament triplet¹⁰⁴ are phosphorylated.

A non-phosphorylated and several phosphorylated variants of desmin and vimentin are present in assembled structures (Z disks, dense body attached and cytoplasmic intermediate filaments) in muscle¹⁰⁵ and non-muscle cells^{100,105,106} while some of the keratin polypeptides are also multiply phosphorylated¹⁴.

Three major activities can be identified in muscle extracts that phosphorylate purified smooth muscle desmin or desmin and vimentin in fibroblastic filamentous cytoskeletons. Two of the activities co-purify with the muscle cyclic AMP-dependent protein kinases and are inhibited by the cyclic AMP-dependent protein kinase inhibitor protein¹⁰⁷. The other activity seems to be cyclic AMP independent¹⁰⁷.

The role of phosphorylation in the regulation of assembly or any other function of intermediate filaments is at present unknown. However, as assembled intermediate filaments are always phosphorylated, phosphorylation may be involved in determining the state of polymerisation or aggregation of these structures.

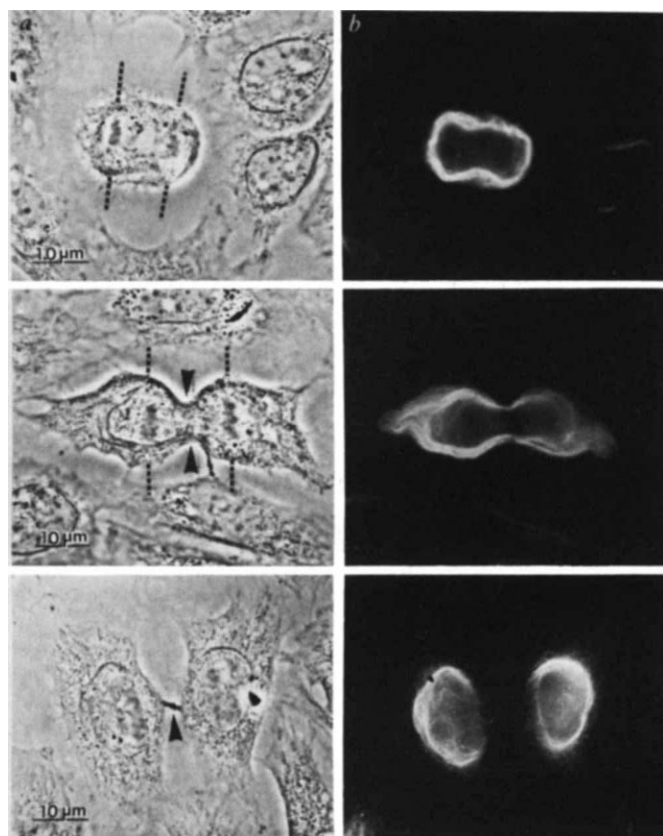


Fig. 6 Endothelial cells during mitosis. Phase (a) and immunofluorescent (b) micrographs using antibodies to vimentin. Scale bars, 10 μ m. Top, anaphase. The dotted lines indicate the position of the chromosomes. Middle, telophase. The contractile ring, indicated by arrowheads in the phase micrograph, begins to pinch the intermediate filaments observed by immunofluorescence. Bottom, late telophase. Cytokinesis has now completely cleaved the ring into two symmetrical crescents that enter the daughter cells. The arrow points to the midbody (from S. Blose, ref. 82).

Prospects for the future

Now that the biochemical and immunological taxonomy of the various intermediate filaments is nearly complete, certain intriguing biological questions arise. The majority of cytoplasmic microtubules and actin filaments emanate from or terminate at one or more nucleation or organisation sites. No such sites have yet been identified for intermediate filaments, but they would have to exist to provide directionality of growth and three-dimensional organisation to these filaments. Keratin filaments are frequently found in association with desmosomes, desmin

filaments with substructures of intercalated disks and vimentin filaments with the nuclear membrane; perhaps these structures can function as nucleation sites for the growth of the respective filaments. In general, intermediate filaments are insoluble in physiological buffers, but highly susceptible to proteolysis, especially in the presence of Ca^{2+} . As these filaments exist mainly in the cytoplasm in a full polymeric state, perhaps their disassembly is regulated by a proteolytic mechanism rather than by a polymerisation-depolymerisation equilibrium as is the case with microtubules. Such a proteolytic mechanism would have an important regulatory role in shape changes that occur during locomotion and cell division.

The co-existence of desmin and vimentin is interesting and it will be important to determine whether the two proteins copolymerise into one and the same filament or co-exist in distinct independent filaments. Skeletal muscle differentiation emerges as a very interesting system to elucidate the proposed function of desmin filaments, the molecular details of the interaction of these filaments with actin and the various types of membranous organelles and the molecular events that lead to their ultimate association with the Z disks. The high degree of keratin polymorphism and the changes in their expression during epidermal differentiation make epidermal differentiation *in vitro* an attractive model system to study the developmental expression of the keratin genes. Finally, although intermediate filaments can be distinguished by immunological methods and peptide mapping, they share a number of properties. To reconcile their morphological similarity and chemical heterogeneity, it is possible to imagine that the subunits of the intermediate filaments are analogous to IgG molecules, namely, that they are composed of a constant and a variable domain. The constant domain might be responsible for the conserved polymerisation of the protein subunits into filaments with an average diameter of 100 Å and their aggregation into filamentous bundles in cells exposed to colcemid. The variable domain might confer on the molecule functional specificity for the cell of which it is a part. Amino acid sequencing data will reveal the validity of this proposition.

Intermediate filaments have emerged as a polymorphic class of cytoplasmic filaments distinct from microtubules and actin filaments. All their biochemical and morphological properties so far indicate that they are involved in mechanically integrating the various components of the cytoplasmic space. As such they should be capable of multiple interactions with cytoplasmic structures, might be expected to have a crucial role in regulation of cell shape and may be influenced by the metabolic state and response to injury or malfunction of any cell in which they are contained.

I thank Dr C. M. O'Connor, B. L. Granger and D. L. Gard for their comments on the manuscript and J. Sauer for drawing Fig. 4. This work was supported by grants from the NIH (GM-06965) and the Muscular Dystrophy Association of America.

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ARTICLES

¹⁸⁷Re–¹⁸⁷Os systematics in meteorites: early chronology of the Solar System and age of the Galaxy

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Using isotope dilution mass spectrometry, we have determined the ¹⁸⁷Re/¹⁸⁶Os, ¹⁸⁷Os/¹⁸⁶Os ratios of five iron meteorites of various groups, and one ordinary chondrite. These results show that iron meteorites and ordinary chondrites were formed within a short time. We determine the ¹⁸⁷Re decay constant as $1.62 \pm 0.03 \cdot 10^{-11} \text{ yr}^{-1}$ and the ¹⁸⁷Os/¹⁸⁶Os isotopic ratio at the origin of the Solar System as 0.805 ± 0.011 . These values are used to compute limits for the age of the Galaxy between 13,300 and 22,400 Myr.

¹⁸⁷Re is one of the long-lived radioactive nuclides with a decay constant (λ) of the order of 10^{-11} yr^{-1} . It produces ¹⁸⁷Os by (β^-) decay. Therefore, like ⁸⁷Rb–⁸⁷Sr or ⁴⁰K–⁴⁰Ar, the ¹⁸⁷Re–¹⁸⁷Os system is a potential cosmochronometer¹. Some peculiar properties of the Re–Os system make it particularly attractive for dating the early formation of the Solar System and for estimating the age of the Galaxy.

(1) Re and Os are refractory siderophile elements that concentrate in the metal and sulphide phases of meteorites². These phases are the dominant material not only in iron meteorites, but also in pallasites and mesosiderites. With regard to ordinary and enstatite chondrites, Re and Os are also mainly contained in the metal phase. Thus Re–Os measurements on this metal phase will give the absolute age of different types of meteorites.

For iron meteorites Re/Os fractionation, being presumably related to the fractional crystallisation of metal in the core of their parent body³, the eventual isochron is expected to date the

formation of irons, that is, the differentiation of parent bodies.

In this way, it would be possible to propose a relative chronology of the early history of the Solar System by comparing the respective ages of, for example, irons and chondrites. Note that in the case of irons this chronometer will lead to an accurate dating, a controversial subject because no other reliable absolute chronometer can be applied to the metal itself: the Rb/Sr is very sensitive to secondary perturbation events⁴, Pb isotopic composition is primordial in iron meteorites⁵, and K–Ar has a limited use because of spallation phenomena.

(2) ¹⁸⁷Re has a half life of the order of magnitude of the age of the Galaxy^{6,7} and is, therefore, potentially a good way of obtaining precise estimates for this age. If ¹⁸⁷Re is produced by (*r*) processes, ¹⁸⁷Os and ¹⁸⁶Os are produced by (*s*) processes only and hence their production rates are not submitted to the uncertainty of the (*r*) process.

Besides these advantages, the ¹⁸⁷Re–¹⁸⁷Os system contains some basic difficulties: first, we do not know the decay constant exactly

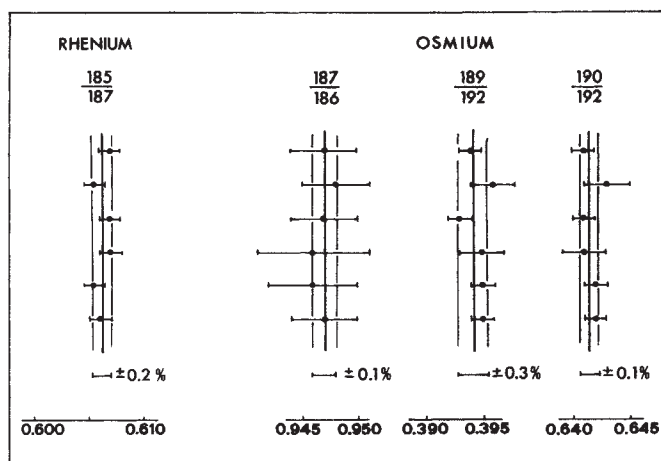


Fig. 1 Reproducibility of ion-probe measurements of standard isotopic compositions of Re and Os.

because its direct measurement by counting methods in the laboratory is very difficult⁸; second, the content of Re and Os in meteorites is quite low (3–0.3 p.p.m.) and the precise isotopic analysis of osmium isotopes is quite difficult at this level.

These difficulties explain why the only serious attempt to use the ^{187}Re – ^{187}Os chronometer in cosmochronology was done by Herr *et al.*¹ and Hirt *et al.*⁹ in the early sixties. The German–Swiss group has approached the above difficulties by determining the rhenium decay constant using terrestrial molybdenites the age of which was approximately known¹⁰. They obtained $1.61 \pm 0.19 \times 10^{-11} \text{ yr}^{-1}$. To overcome the second problem they used gas source mass spectrometry but their technique was neither very sensitive nor precise. Using their previously determined decay constant, they obtain for iron meteorite samples from several groups an age of 4,000 Myr.

We have used a more refined technique for Re and Os measurements, considered a new set of iron meteorites plus one chondrite, and recalculated the age of the Galaxy.

Experimental technique

We have used, both for Re and Os, isotope dilution mass spectrometry, using ^{185}Re and ^{190}Os spikes prepared by Oak Ridge National Laboratories. After dissolution and spiking Re and Os are separated chemically, then loaded on an aluminium disk and run separately in an ion-sputtering mass spectrometer. The separation between Os and Re is absolutely crucial because they interfere at mass 187—the mass of interest.

Chemistry: The details of the technique will be published elsewhere and only the principles are described here.

Metal phases are dissolved in 6M H_2SO_4 and at the same time ^{185}Re and ^{190}Os spikes are added. Oxidation is carried out through an excess of CrO_3 and osmium is distilled at 110 °C as OsO_4 into a cooled water trap. After transforming the oxide to bromide then to chloride, Os is loaded on an anion-exchange column for purification. It is eluted with 4M HNO_3 and then loaded on a 99.99% pure aluminium disk.

Re is extracted from the distillation residue by tribenzylamine in chloroform and then back extracted with ammonia into aqueous solution. It is loaded on an anion-exchange column, eluted with 8M HNO_3 and then loaded on a pure aluminium disk.

Mass spectrometry: The Cameca SMI300 ion probe was used as a sputtering mass spectrometer. The sputtering is done by a negative oxygen ion beam. The target is the Os or Re deposit on the aluminium disk. The machine which was largely modified for our experiments has been described elsewhere¹¹. It is equipped with an energy filtering system through an electrostatic analyser and a programmable magnetic field and digital output. Energy filtering eliminates most of the possible interfering species including hydrocarbons.

Performances: The total blanks of our chemistry are: for Re < 3ng; for Os < 5ng. At such a low level the measurements are imprecise due to small interferences; we have, therefore, only estimated the possible upper limits. In fact, blanks are probably much lower. The reproducibility of isotopic ratio measurements in the mass spectrometer is $\pm 0.2\%$ for Re, $\pm 0.1\%$ for Os. The standard runs are illustrated in Fig. 1. A duplicate analysis has been done on the iron meteorite Tlacotepec to estimate whether isotope dilution was achieved:

Experiment 1	Re = 3.69 ± 0.03 p.p.m.
	Os = 57.8 ± 0.3 p.p.m.
Experiment 2	Re = 3.29 ± 0.02 p.p.m.
	Os = 57.7 ± 0.8 p.p.m.

These results show an extremely good reproducibility.

Meteorite chronology

As established by Hirt *et al.*¹⁰ we can write the chronometric equation for a closed system using ^{186}Os as reference isotope.

$$\left(\frac{^{187}\text{Os}}{^{186}\text{Os}} \right)_{\text{Today}} = \left(\frac{^{187}\text{Os}}{^{186}\text{Os}} \right)_{T_0} + \left(\frac{^{187}\text{Re}}{^{186}\text{Os}} \right)_{\text{Today}} (e^{\lambda T_0} - 1)$$

where T_0 is the age at the closure of the system.

This equation is then used to compare $^{187}\text{Os}/^{186}\text{Os}$ with $^{187}\text{Re}/^{186}\text{Os}$ to determine the age of the system, the initial isotopic ratio of osmium, and at the same time to check if the subsystems are cogenetic and closed. We have thus analysed several iron meteorites of different classes: Canyon Diablo (IB), Coahuila (IIA), Henbury (IIIA), Casas Grandes (IIIA), Tlacotepec (IVB) and one ordinary chondrite, St Séverin (LL6). For each meteorite the iron part has been taken (separated in the case of St Séverin) and treated as described above. The results, given in Table 1 and Fig. 2, show a wide range of Re/Os values.

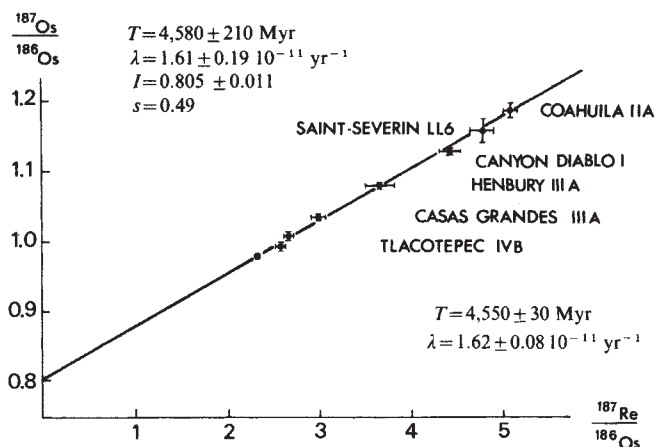


Fig. 2 ^{187}Re – ^{187}Os evolution diagrams for five different iron meteorites from four different groups and one chondrite. s is the reduced χ^2 value and indicates the excellent fit to a straight line.

From both our isotopic measurements and from expected $^{187}\text{Os}/^{186}\text{Os}$ ratios for irons of various classes², it seems that the Re/Os ratio and, hence the radiogenicity of osmium, could depend on the class to which the iron belongs. That is, in general, meteorites from class I are expected to be more radiogenic than those from class IIIA, and less than that from class IIA. This slight trend could be related to the oxidation state of irons. However, the same data do not presently suggest any trend within a given group. In this case, the Re and Os fractionation might involve another factor besides the degree of fractional crystallisation of the metal phase. More measurements are needed to confirm these results.

Regarding the measurements made on the iron meteorites, all points fall remarkably on a single line defining an extremely good isochron ($s = 0.49$). The slope is 0.0765 ± 0.0035 and the initial ratio $^{187}\text{Os}/^{186}\text{Os} = 0.805 \pm 0.011$ (2σ errors). These two results differ substantially from the results of Hirt *et al.*⁹ for the slope, as well as for the initial ratio and the precision (respectively 0.066 ± 0.013 and 0.83 ± 0.06). However, this difference is the normal difference which can be expected from almost 20 years of analytical progresses in the field: note that all our results are within the error limits given by the German-Swiss group. Using the decay constant $\lambda_{\text{Re}} = 1.61 \times 10^{-11} \text{ yr}^{-1}$, we obtain an age of $4,580 \pm 210 \text{ Myr}$.

Several conclusions can be drawn from these results.

First, within the accuracy of our measurements, the iron meteorites of several groups were formed with a short time interval with ordinary chondrites. Such an unequivocal result has not been clearly established before, except by I-Xe chronology^{12,13}. The Rb-Sr approach of the Caltech group gave various ages from 4,700 to 3,800 Myr and no clear answer to this question¹⁴. However, all the precedent results dated the silicate inclusions, not the iron itself. Our results show that if iron meteorites come from differentiated bodies, such a differentiation occurred within a period of about 400 Myr in their respective parent bodies.

Second, the time interval between these events and the well determined formation of ordinary chondrites is very short—say $< 200 \text{ Myr}$; this value is obtained from the extreme ages for St Séverin (error limits) compared with the 4,580 Myr age for iron meteorites. Indeed as most, if not all, of Re and Os from chondrites is contained in their metallic part¹⁵, the determination for Saint Séverin can be considered as that for the whole rock of this unperturbed ancient meteorite¹⁶. In other words, the differentiation of planetary bodies is a very early event in the Solar System. Such an early differentiation is consistent with the existence of early magmatic activity in the Solar System as determined on the Juvinas basaltic achondrite¹⁷.

From our results we can deduce a decay constant for the ^{187}Re . The recent results from our group clearly establish the age of formation of basaltic achondrites^{17,18}, H chondrites¹⁹, enstatite chondrites²⁰, and LL chondrites^{16,19} both by Rb-Sr and U-Pb methods²¹ in the range of $4,550 \pm 30 \text{ Myr}$. Such results are also in good agreement with the data obtained by Turner *et al.*²² using the ^{39}Ar - ^{40}Ar method, and by Lugmair using the Sm-Nd chronometer²⁸. Taking the age as $4,550 \pm 30 \text{ Myr}$, we obtain $\lambda_{\text{Re}} = 1.62 \pm 0.08 \cdot 10^{-11} \text{ yr}^{-1}$. Note that such a value is extremely close to the previous value of $1.61 \cdot 10^{-11} \text{ yr}^{-1}$ determined by Hirt *et al.*¹⁰ on terrestrial molybdenite samples. We stress again the remarkable results obtained by these authors 20 years ago.

Age of the Galaxy

We will use the values of λ_{Re} and ($^{187}\text{Os}/^{186}\text{Os}$) initial ratio to determine the so-called age of the Galaxy following at first an early approach by Clayton⁶.

^{186}Os and ^{187}Os isotopes are created by (*s*) nucleosynthetic processes. In addition, ^{187}Os contains a radiogenic contribution from ^{187}Re . On the other hand, ^{187}Re is created by (*r*) process. Therefore

$$^{186}\text{Os} = ^{186}\text{Os}(s)$$

$$^{187}\text{Os} = ^{187}\text{Os}(s) + ^{187}\text{Os}^*$$

Where $^{187}\text{Os}^*$ is the radiogenic contribution. On the other hand,

$$\frac{d^{187}\text{Os}^*}{dt} = \lambda_{\text{Re}} \times ^{187}\text{Re}$$

and

$$\frac{d^{187}\text{Re}}{dt} = A(t) - \lambda_{\text{Re}} ^{187}\text{Re}$$

where $A(t)$ is the (*r*) process production rate for ^{187}Re .

Following Clayton⁶, we can take $A(t)$ in the form of $A_0 e^{-\beta t}$. When β is large, one obtains the limiting case of sudden nucleosynthesis. When β is small one obtains a steady-state nucleosynthesis.

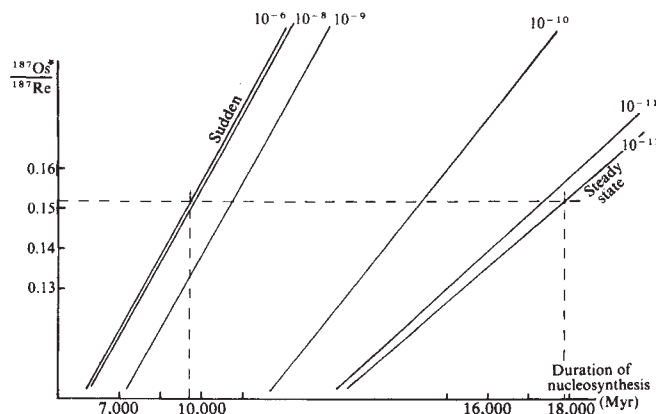


Fig. 3 Variation of the $^{187}\text{Os}^*/^{187}\text{Re}$, at the formation of the Solar System, with respect to the duration of nucleosynthesis, for different time constants (β) of the nucleosynthesis production rates.

The integration of the above differential equations give

$$\frac{^{187}\text{Os}^*}{^{187}\text{Re}} = \frac{\lambda/\beta(1 - e^{-\beta\Delta t}) - (1 - e^{-\lambda\Delta t})}{(e^{-\beta\Delta t} - e^{-\lambda\Delta t})} = \psi(\Delta t)$$

at the formation of the Solar System, when the solar nebula became isolated from the Galaxy. Δt is the time between starting of nucleosynthesis and meteorite formation, that is, the age of the Galaxy before the formation of the Solar System. Note that A_0 does not play any part in this equation which is a remarkable property of parent-daughter cosmochronology.

Taking as usual ^{186}Os as the reference, we obtain

$$\frac{^{187}\text{Os}^*}{^{186}\text{Os}} / \frac{^{187}\text{Re}}{^{186}\text{Os}} = \psi(\Delta t)$$

But on (*s*) process we know that $^{187}\text{Os}(s)\sigma_{^{187}\text{Os}} \sim ^{186}\text{Os}(s)\sigma_{^{186}\text{Os}}$ where $\sigma_{^{187}\text{Os}}$ is the neutron capture cross-section of ^{187}Os .

$$\frac{^{187}\text{Os}^*}{^{186}\text{Os}} = \frac{^{187}\text{Os}}{^{186}\text{Os}_{\text{total}}} - \frac{\sigma_{^{186}\text{Os}}}{\sigma_{^{187}\text{Os}}} \cdot f$$

$$\frac{^{187}\text{Re}}{^{186}\text{Os}} = \left(\frac{\text{Re}}{\text{Os}} \right)_{\text{atomic}} \cdot \phi$$

where ϕ is the abundance of ^{187}Re in Re over the abundance of ^{186}Os in Os. The cross-sections (Maxwell averaged) were recently measured by Browne and Berman²³ for $kT = 30 \text{ keV}$.

$$\sigma_{^{186}\text{Os}}/\sigma_{^{187}\text{Os}} = 0.39 \pm 0.03$$

f is a correction factor which takes into account the dependance of the cross-section on the temperature and the possible excited state of the atoms in stars. Woosley *et al.* (in ref. 7) give $f = 0.82$. The (Re/Os) ratio in carbonaceous chondrites which are supposed to represent the best estimate of the Solar System average value is 0.081 (ref. 24). The fact that this ratio does not vary too much between different meteorite classes^{15,25,26}, and the Earth²⁷ indicates that this value is probably quite constant cosmochemically speaking. From our measurements, we calculate $\phi = 40.88$, then $^{187}\text{Re}/^{186}\text{Os} = 3.20$, and $^{187}\text{Os}^*/^{187}\text{Re} = 0.152$.

Table 1 $^{187}\text{Re}/^{186}\text{Os}$ and $^{187}\text{Os}/^{186}\text{Os}$ ratio for iron meteorites and the magnetic separate from St Séverin

Sample		Sample weight (mg)	Re (p.p.m.)	Os (p.p.m.)	$^{187}\text{Re}/^{186}\text{Os}$	$^{187}\text{Os}/^{186}\text{Os}$
<i>Iron meteorites:</i>						
Tlacotepec	no. 1	187.3	3.28 (3)	57.0 (4)	2.32 (3)	0.980 (4)
(IVB)	no. 2	264.7	3.69 (3)	57.8 (7)	2.56 (5)	0.994 (6)
	no. 3	253.6	3.69 (3)	56.0 (6)	2.65 (5)	1.008 (6)
Canyon Diablo	IA	4075.0	0.236 (1)	2.12 (2)	4.47 (5)	1.122 (8)
Coahuila	IIA	411.9	1.40 (1)	11.11 (6)	5.06 (7)	1.187 (11)
Casas Grandes	IIIA	2446.9	0.731 (6)	9.87 (14)	2.98 (7)	1.035 (5)
Henbury	IIIA	472.0	1.53 (5)	16.9 (2)	3.63 (16)	1.080 (4)
<i>Chondrite:</i>						
St Séverin						
magnetic separate	LL6	636.8	0.939 (8)	8.00 (18)	4.72 (16)	1.164 (15)

The numbers in parentheses are the errors on the results, expressed in last digits: for example, 3.28(3) is equivalent to 3.23 ± 0.03 and 3.63(16) is equivalent to 3.63 ± 0.16 .

We can then compute for these different values of (β) the curves showing the variation of $^{187}\text{Os}^*/^{187}\text{Re}$ with Δt (duration of nucleosynthesis (Fig. 3). Choosing for β the extreme values

10^{-6} yr^{-1} for sudden nucleosynthesis

10^{-12} yr^{-1} for steady state nucleosynthesis

we obtain for $^{187}\text{Os}^*/^{187}\text{Re} = 0.152$ two intercepts that give us an interval for the duration of nucleosynthesis: $8,700 < \Delta t <$

17,800 Myr and hence the age of the Galaxy is $13,300 < T < 22,400$ Myr. Note that a $\pm 10\%$ variation on $^{187}\text{Os}^*/^{187}\text{Re}$ would lead to a $\pm 8\%$ variation on T . Such an uncertainty might come from the cosmic Re/Os ratio or from the factor f or from the neutron capture cross-section ratio.

We thank G. Manhes and J. F. Minster for discussions, A. Pierre for technical assistance and P. Pellas for a magnetic separate from Saint Séverin.

Received 28 September; accepted 20 November 1979.

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Bursts of aftershocks, long-term precursors of strong earthquakes

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Earthquakes that are followed within a short time by abnormally large numbers of aftershocks are hypothesised to be long-term precursors of stronger earthquakes. In a test of the hypothesis, 18 out of 23 strong earthquakes, in five regions worldwide, were predicted retrospectively. In comparison with a random model the precursory pattern occurs at a satisfactorily high confidence level.

THE hope that patterns of seismicity exist that are premonitory to strong earthquakes relies on the fact that the occurrence of earthquakes is controlled by the distribution of stresses and strengths within the Earth and that each earthquake in turn changes these fields. At present we cannot anticipate what such patterns should be, because the theoretical basis is inadequate. One reason for this is the absence of firmly established empirical

regularities in seismicity which would provide a factual basis for theoretical studies of earthquake occurrence.

Searches for premonitory patterns of seismicity have attracted much attention, as these may possibly be established by the analysis of earthquake catalogues which are the longest record of the relevant observations. With regard to precursors of large earthquakes, there have been many purported descriptions of

premonitory patterns of seismicity. These patterns are of three basic types—quiescence, activation and migration of epicentres. Although the results from some of these studies support the hope that premonitory patterns of seismicity exist, nevertheless, with a few exceptions, their definition is not specific enough to estimate the reliability of the pattern. To obtain such estimates one should be able to detect the pattern by a formal algorithm and to count how often it is successful as a precursor. Without such estimates of reliability, any premonitory pattern would be uncertain.

We present here an attempt to derive a formal definition of some premonitory seismicity patterns from the analysis of an earthquake catalogue, which yields a reasonable success-to-failure ratio in retrospective long-term earthquake prediction. The definition also provides constraints on the theory of earthquake occurrence.

One reason why the search for premonitory patterns of seismicity has not been successful is that aftershocks are usually disregarded as being highly predictable phenomena. The results we describe below show that, while aftershocks are indeed highly predictable, some specific traits of aftershock sequences are highly informative. These traits may represent important, descriptive aspects of the distribution and redistribution of stresses and strengths within the Earth.

Definitions

Let t be the origin time of an earthquake, λ , ϕ the longitude and latitude of its epicentre (west and south are negative), h its focal depth, and M its magnitude. The subscript j will indicate the sequence number of an earthquake in the catalogue, $t_i < t_{j-1}$.

We seek precursors of earthquakes with magnitudes $M \geq M_0$. These are called strong earthquakes. Our patterns will be constructed from earthquakes in the magnitude range $M_2 \equiv M_0 - \mu_2 \leq M \leq M_0 - \mu_1 \equiv M_1$ and in a time interval of duration s ; the values of μ_1 , μ_2 and s may be different for each of the three patterns that we will discuss.

Aftershocks are defined as follows. Consider two earthquakes with sequence numbers i and j , $j > i$. The second earthquake is an aftershock of the first if the following conditions are satisfied: the distance between their epicentres is less than $R(M_i)$; $t_j - t_i \leq T(M_i)$; $h_j - h_i \leq H(M_i)$; $M_j \leq M_i$. Here $T(M)$, $R(M)$ and $H(M)$ are empirical functions. If we apply this definition to an earthquake catalogue, starting with the first earthquake, we can separate the catalogue into main shocks and their aftershocks. The first earthquake of the catalogue is identified as a main

shock. Each succeeding main shock can be identified after the deletion of aftershocks of the preceding main shocks. Formulas of this type have been developed¹⁻³ for both the worldwide and the southern California⁴ catalogues for use in other aspects of the study of aftershocks and sequences or chains of inter-dependent earthquakes.

Pattern B (burst of aftershocks). Let $b_i(e)$ be the number of aftershocks following the i th main shock within time interval e . We consider only those main shocks with magnitudes between M_2 and M_1 and only aftershocks with magnitudes at or above some threshold $m \equiv M_0 - \mu_3$. We invoke the thresholds M_2 and m for practical reasons as all catalogues are incomplete and inhomogeneous for small magnitudes. However, these thresholds may be of some value in explaining physical relationships, as the details of premonitory patterns may be different in different magnitude ranges.

Pattern B consists of a main shock and its aftershocks for which $b_i \geq C$. A more flexible definition of a threshold for b_i can be given⁵, but it is not required for the present purposes.

An alarm is declared for a period of τ years after pattern B is diagnosed. A strong earthquake is expected during this period, having been predicted by pattern B. The alarm is terminated either at the time a strong earthquake takes place or at the end of this period, whichever comes first.

Pattern S ('swarms') was introduced elsewhere and its parameters were adjusted to the Italian earthquake catalogue⁶. We count the number of earthquakes in a time-window from $(t-s)$ to t , in the same magnitude range as above and in a restricted depth range. Let $N(t)$ be the total number of such earthquakes in the region. A reduced number of earthquakes $n(t)$ is obtained from $N(t)$ by the elimination of aftershocks of strong earthquakes. We then consider the map of the epicentres that are counted to determine $n(t)$. From this map we determine a third function $r(t)$ which is the maximum number of epicentres that can be surrounded by a small rectangular cell of dimensions $\Delta\phi$ in latitude and $\Delta\lambda$ in longitude.

Pattern S consists of a group of earthquakes such that $n(t) > \max\{C_1, C_2 N(t)\}$ and $r(t) > C_3 n(t)$. $\bar{N}(t)$ is the average of $N(t)$ over the interval t_0 to t or from $(t-ks)$ to t . The threshold M_1 plays no part in the identification of this pattern.

Pattern Σ has been introduced elsewhere⁷ and its parameters were adjusted to some of the strongest earthquakes of the world. It is represented by a peak of the function $\Sigma(t) = \sum_j G(M_j)$ where the summation takes place over the same band of time and magnitude intervals as above. Aftershocks of strong earthquakes are eliminated from the summation. $G(M)$ is specified⁷

Table 1 Score of patterns B

Regions	1 Southern California	2 N. California- Nevada (excluding Mendocino area)	3 Southern Japan	4 Northern Japan	5 New Zealand	Totals
Catalogue ref.	4, 11, 12	9	10, 14	10, 14	13	
Span of catalogue (yr)	1932-78	1945-76.5	1926-76.5	1940-76.5	1945-77	201.5
M_0	6.5	6.2	8	7.6	6.6	
C	11-13	6-12	4-10	4-6	9-14	
Strong earthquakes						
total	6	4	2	4	7	23
'predicted'	5	3	2	4	4	18
Relative time of alarms	0.46	0.27	0.18	0.36	0.27	0.31
N_m	36	39	9	45	65	194
N_A/N_B	9/14	3/6	2/4	7/13	7/8	28/45
T_τ/T	0.37	0.28	0.10	0.27	0.53	0.30
P	96%	79%	95%	96%	95%	

N_m , total number of main shocks; N_B , number of main shocks which generate pattern B $b_i \geq C$, for upper value of C ; N_A , number of occurrences of pattern B which are followed by strong earthquakes within τ yr; T_τ , sum of times τ before each strong earthquake, counting overlapping parts only once; T , span of the catalogue (yr); C , threshold for identification of pattern B. A variation of C within the limits indicated in the table will introduce either zero or one additional error; P , confidence level for upper value of C .

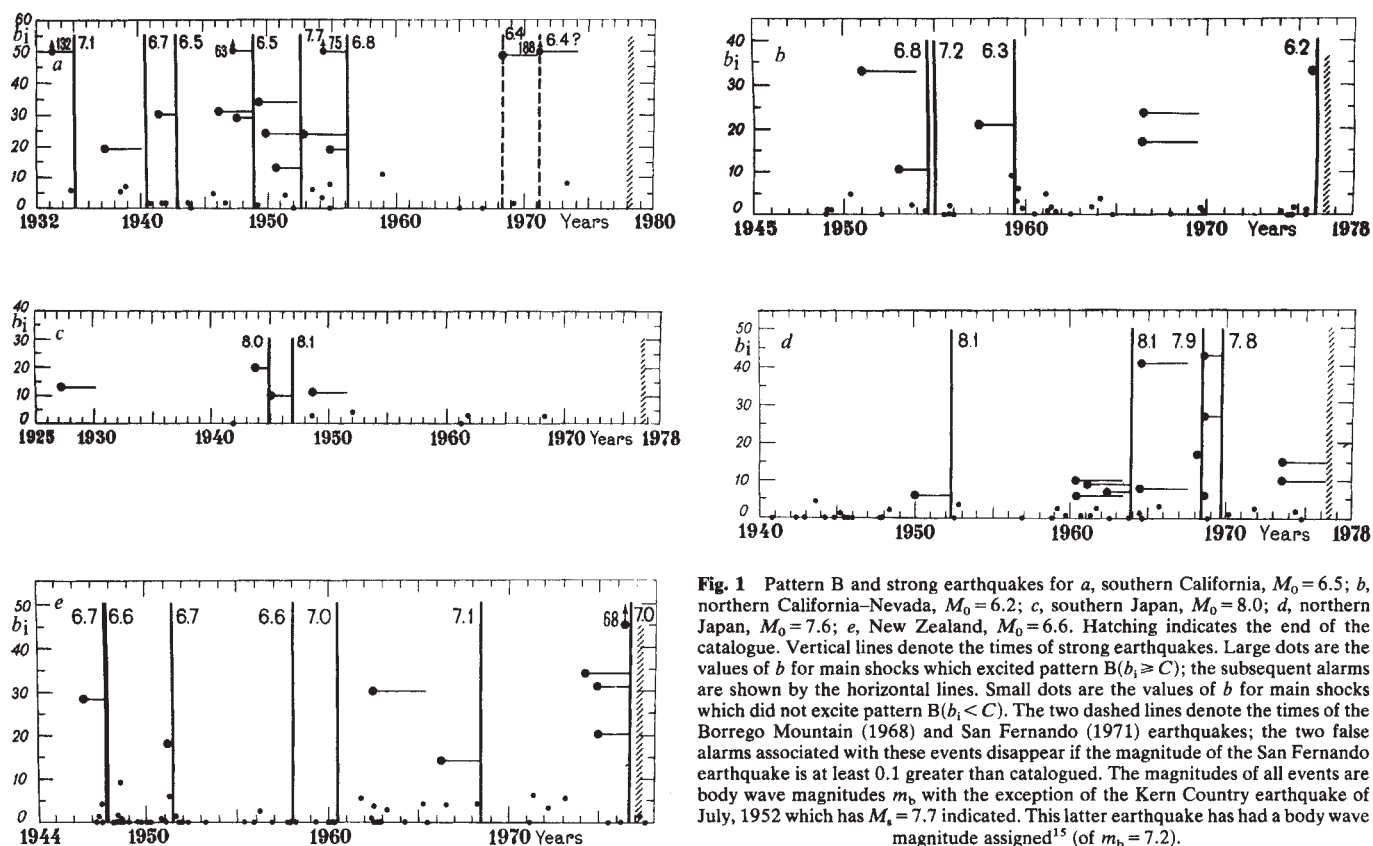


Fig. 1 Pattern B and strong earthquakes for *a*, southern California, $M_0 = 6.5$; *b*, northern California-Nevada, $M_0 = 6.2$; *c*, southern Japan, $M_0 = 8.0$; *d*, northern Japan, $M_0 = 7.6$; *e*, New Zealand, $M_0 = 6.6$. Hatching indicates the end of the catalogue. Vertical lines denote the times of strong earthquakes. Large dots are the values of b for main shocks which excited pattern B ($b_i \geq C$); the subsequent alarms are shown by the horizontal lines. Small dots are the values of b for main shocks which did not excite pattern B ($b_i < C$). The two dashed lines denote the times of the Borrego Mountain (1968) and San Fernando (1971) earthquakes; the two false alarms associated with these events disappear if the magnitude of the San Fernando earthquake is at least 0.1 greater than catalogued. The magnitudes of all events are body wave magnitudes m_b with the exception of the Kern County earthquake of July, 1952 which has $M_b = 7.7$ indicated. This latter earthquake has had a body wave magnitude assigned¹⁵ (of $m_b = 7.2$).

to be $G(M) = 10^{d(M-f)}$ where d and f are numerical parameters. Pattern Σ consists of earthquakes satisfying the condition $\Sigma(t) \geq \bar{\Sigma}$. The values $\bar{\Sigma} = cG(M_0)$, $c = 0.5$, $d = 0.91$ have been assumed elsewhere⁷.

Alarms after patterns S and Σ are declared in the same manner as indicated after pattern B.

Data processing

We have tested pattern B in a retrospective 'prediction' of strong earthquakes in the five regions indicated in Table 1. Arbitrary decisions have had to be made regarding the definition of the geographical boundaries of these regions as well as the values of all parameters. No *a priori* theoretical limits can be imposed on these decisions and we have to obtain them by data fitting. This creates a danger of self-deception as the number of these decisions is rather large. To decrease this danger we have tried to make these decisions simple, uniform and, if possible, *a priori*. Also we have accepted, when possible, the arbitrary choices of parameters or thresholds made in previous studies, and we have tested the stability of our results with regard to variations in each of these choices. We have considered only those earthquakes with $h \leq 100$ km.

We have assigned the following values: $\tau = 3$ yr; $e = 2$ days; $\mu_1 = 0.1$; $\mu_2 = 1$; $\mu_3 = 3.5$. These values were obtained by data fitting for southern California⁵. To define aftershocks we took $R = 50$ km; $T(M) = 0.5$ years for $5.0 \leq M \leq 5.4$, $T(M) = 1$ yr for $5.5 \leq M \leq 6.4$ and $T(M) = 2$ yr for $M \geq 6.5$. The values of $R(M)$, $T(M)$ given elsewhere³ for the southern California catalogue give better results, but these fine gradations are hardly warranted in view of the accuracy of some of the other catalogues used in this study. The values of $R(M)$ and $T(M)$ assumed here have the advantage that they can be used for a fast diagnosis of pattern B even before the epicentres of aftershocks are determined precisely.

The values of M_0 and C listed in Table 1, have been chosen separately for each region due to lack of experience and a satisfactory theory. The fact that these two parameters have

been chosen *a posteriori* contributes the single largest difficulty in the evaluation of the significance of our results.

We have chosen the other parameters to be the same for all regions, even if they are unreasonably simple. This suffices for our present purpose which is to test the existence and stability of pattern B. For actual predictions, the parameters could probably be better adjusted to match the specific geophysical characteristics of the region as well as its own earthquake catalogue. More detailed studies of pattern B in some specific regions will be published elsewhere⁸.

The boundaries of the southern California as well as the northern California-Nevada regions are defined elsewhere^{4,9}. We straightened these boundaries slightly from the original definitions, and have deleted the area around Cape Mendocino from the northern California study as it has a distinctly isolated cluster of epicentres. The boundaries of the other regions are based on large-scale neotectonics and on the densities of epicentres.

Results

The results are summarised in Table 1. Examples of the details are documented in Table 2 and displayed in Fig. 1. The total span of all five catalogues is 202 yr. Twenty-three strong earthquakes occurred in the five regions during these time intervals. Eighteen of these earthquakes were retrospectively predicted, that is, they took place during the intervals of alarms sounded after each instance of pattern B. The total duration of these alarms is 60 yr or 30% of the total span of the catalogues. If we exclude the results for southern California, which were highly involved in the data fitting, that is, in the adjustment of parameters, we have 13 out of 17 strong earthquakes predicted for the remaining four regions; the total duration of alarms is 39 out of 155 yr or 25% of the total time.

Although these numerical values illustrate the practical efficiency of pattern B as a tool in prediction, they cannot be used in statistical tests of the existence of pattern B as, by the definition above, an alarm is terminated by the occurrence of a

strong earthquake. To perform this statistical test (of the existence of pattern B), the values of the three parameters, N_A , N_B , and T_e/T were tabulated and listed in Table 1.

The probability that a random binomial process could do as well or better than pattern B in anticipating strong earthquakes is given by the quantity $(1 - P)$. This is the probability that N_A events or more will occur out of N_B tries in the fraction T_e/T of the total catalogue time T according to a random binomial process. The values of P strongly support the hypothesis that B is a premonitory pattern, although these values of probability should only be used in a qualitative sense rather than literally. The fact that some thresholds were chosen *a posteriori* leads to an overestimation of P . On the other hand, the fact that some thresholds are chosen to be the same for all regions, leads to an underestimation of P . For example, the earthquake in southern Japan of 28 June 1948 could be deleted as a cause of a false alarm (Table 2) either by (1) changing e from 2 to 7 days, and/or (2) changing m to 5.0 from 4.5. Both of these changes are not unreasonable: in the first instance because $M_0 = 8$ is especially large and in the second because the catalogue¹⁰ is more reliable for magnitudes greater than 5 than it is for magnitudes between 4.5 and 5. The last two false alarms for the southern California catalogue (Fig. 1) disappear if we assume that the magnitude of the San Fernando earthquake of 9 February 1971 is 6.5 instead of 6.4; this question is discussed elsewhere⁵. Similarly, the values of P could be increased by adjusting some of the parameters to take into account specific traits of the observed seismicity for each region separately. However, these adjustments would be non-unique; for the purpose of testing the existence of pattern B as a precursor, it is better to keep the criteria for all regions as uniform as possible, and that we make

them, insofar as possible, *a priori*, and hence avoid tampering with the results by intuitive 'improvements' after inspection of the tabulations.

In the case of the northern California-Nevada catalogue we took $m = 3$, instead of $m = 2.7$ which would have been obtained from a literal application of our uniform definition $\mu_3 = 3.5$; this catalogue is especially inhomogeneous for $m < 3$. The errors in prediction with $m = 2.7$ and $m = 3$ are the same.

Table 2 lists the strong shocks as well as the main shocks that trigger pattern B; the strong shocks are underlined. Thus it is possible to see whether or not a strong shock was preceded by pattern B within 3 yr. Table 2 shows that our results remain essentially unaltered when the value of R is changed to 100 km and/or e to 7 d. Similar results have been obtained for the other catalogues.

We have indicated in Table 2 those events that would have been identified as aftershocks of the event immediately preceding had we used $R = 100$ km; deletion of these events from our listing would have improved our success rate as these invariably result in a reduction in N_B without a corresponding decrease in N_A . There are two examples of entries of this type in the southern California catalogue and none in the northern California and New Zealand catalogues.

A more detailed analysis of the stability of pattern B as well as the choice of the optimum parameters for the diagnosis of pattern B requires a separate study for each region. We have tried here to emphasise that pattern B can score quite satisfactory success-to-failure ratios, even with parameters that are uniform for all regions, despite the fact that each region has widely different tectonics, seismicity and quality of earthquake catalogue from the others.

Table 2 Pattern B and strong earthquakes

Month-day-year	epicentre		M	b_i		δT (yr)	n_m
	ϕ	λ		$R = 50(100) \text{ km}$			
				$e = 2 \text{ d}$	$e = 7 \text{ d}$		
Southern Japan							
Polygon: (30; 130), (33; 139), (39; 136), (33; 127.5)							
3- 7-27	35.6	135.1	7.5	13	18	7.8	3
9-10-43	35.5	134.2	7.4	20	25	1.24	
12- 7-44	33.7	136.2	8.0	3	3		
1-13-45	34.7	137.0	7.1	10	17	1.94	1
12-21-46	33.0	135.6	8.1	2	3		
6-28-48	36.1	136.2	7.3	11	11	≥ 30	
Northern Japan							
Polygon: (33; 139), (37; 145), (40; 146), (45; 154), (49; 150), (45; 140), (39; 136)							
12-26-49	36.7	139.7	6.7	6	7	2.19	14
3- 4-52	42.2	143.9	8.1	6(21)	9(28)		
3-21-60	39.8	143.5	7.5	10(13)	19(30)	3.56	
3-23-60	39.3	143.8	6.7	6	6	3.56	*
1-16-61	36.0	142.3	6.8	9(13)	9(13)	2.74	
4-12-62	38.0	142.8	6.8	7(8)	10(12)	1.50	
10-13-63	43.8	150.0	8.1	4(8)	9(16)		15
5- 7-64	40.3	139.0	6.9	8	10	4.03	
6-16-64	38.4	139.2	7.5	41(42)	48(52)	3.92	
1-29-68	43.2	147.0	6.9	17(21)	23(33)	0.30	6
5-16-68	40.7	143.6	7.9	12(45)	17(65)		
5-16-68	41.4	142.8	7.5	27	50	1.24	
5-17-68	39.8	143.5	6.7	6	7	1.24	*
6-12-68	39.4	143.1	7.2	43(45)	51(55)	1.17	
8-12-69	42.7	147.6	7.8	7(37)	18(66)		
6-17-73	43.0	146.0	7.4	15(30)	18(45)	>4	4
6-24-73	43.0	146.8	7.1	10(30)	16(45)	>4	
							*

Values of b_i for $R = 100$ km are indicated only if they are not the same as for $R = 50$ km; δT , time from the given pattern B until the next strong earthquake; n_m , the number of all main shocks between the given and preceding strong earthquakes, or since the beginning of the catalogue in the case of the first strong earthquake; * denotes a main shock, that would have been identified as an aftershock with $R = 100$ km.

A speculation on generalisation

The same catalogues were processed to detect the presence of patterns S and Σ (Fig. 2). The value of M_0 is assumed to be the same as for pattern B. As in the papers where these patterns were introduced^{6,7}, the parameters were taken to be as follows: $C_1 = 0$, $C_2 = 1$, $C_3 = 0.5$, $\mu_2 = 2$, $\mu_1 = 0.5$ for Σ (absent for S), $d = 0.91$, $f = 4.5$, $s = 1$ yr for S. For Σ we varied $\bar{\Sigma}$ and s for each of the regions (Fig. 2). The absence of a pattern signifies that it could not be detected for the given region. We added data for Alaska in view of the striking success of pattern Σ .

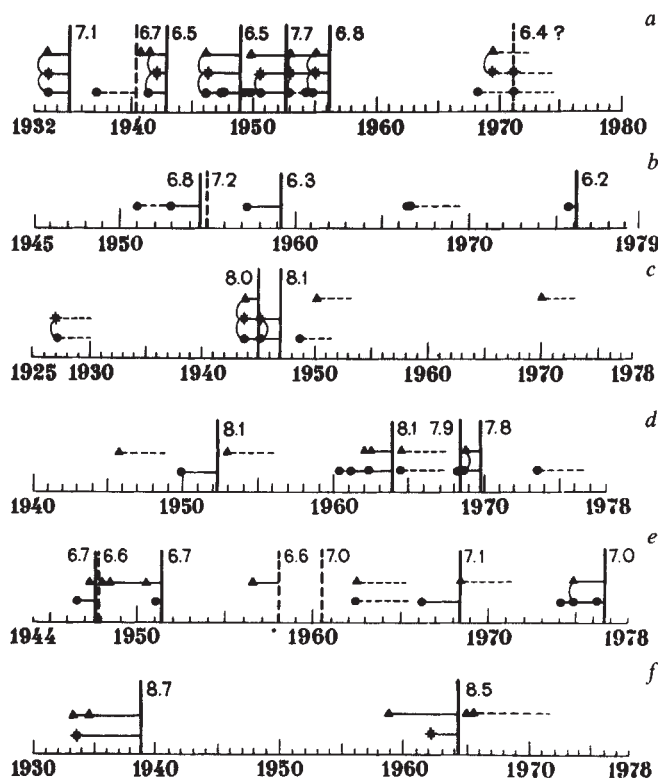


Fig. 2 Occurrence of patterns B (●), S (★) and Σ (▲).

	$\bar{\Sigma}$	s (yr)	$\Delta\phi$	$\Delta\lambda$
a, Southern California	60	1	0.4°	0.6°
b, Northern California-Nevada				
c, Southern Japan	900	3	0.4	0.6
d, Northern Japan	700	1	1.0	1.4
e, New Zealand	95	0.5	0.4	0.6
f, Alaska	770	2	0.5	1.0

Solid horizontal line is period of alarm; dashed is false alarm. Solid vertical line is time of strong earthquake; dashed is strong earthquake not predicted by pattern B. Brackets denote different patterns generated by the same main shock and the appropriate parts of its aftershock series.

In several cases the same main shock and its aftershocks will give rise to several different patterns from among Σ , S, B. This result is not unreasonable as by definition, a main shock and its aftershocks will generate:

- (1) pattern B if the number of aftershocks during the first e days is large enough;
- (2) pattern S if the number of aftershocks during the time interval s , or during any part of it, is large enough; we usually take $s = 1$ yr;
- (3) pattern Σ if a sufficiently large number of events have magnitudes close to the upper limit M_1 .

In most cases in southern California the same main event plus its aftershocks generate two or even all three patterns. Moreover, the coincidence of a combination of two or three of the patterns eliminates false alarms without adding any failures-to-predict⁵.

Patterns Σ and S also appear in conjunction before extremely strong earthquakes in the Tibet-Himalaya region⁸; in this region pattern B cannot be diagnosed, as the catalogues that are available do not include aftershocks.

We find that prediction by pattern B is successful when two conditions are satisfied: not only is a large number of aftershocks b_1 required but also the main shock producing it must have a large enough magnitude; if the magnitude of a main shock is too low, but is still followed by an exceedingly large b_1 , a false alarm will be generated. We have therefore introduced the threshold M_2 . On the other hand we find both the numbers of false alarms and failures-to-predict increase significantly if we ignore the number of aftershocks produced by a main shock and merely consider the occurrence of a main shock itself as a possible precursor: for example, these numbers increased when we considered as a premonitor any earthquake with magnitude up to M_0 , that is, by removing the gap between M_2 and M_0 , by increasing M_1 and ignoring the threshold C .

We can imagine that a hypothetical generalised premonitory pattern exists which is an abnormal clustering of earthquakes in a space-time-energy domain. We call this hypothetical pattern a 'burst of seismicity'. Our three particular patterns may represent different projections of this generalised pattern. The definitions of the three are neither unique nor optimised; the use of discrete magnitude thresholds, especially in regard to large uncertainties in magnitudes of earthquakes in the catalogues, is an inadequate and troublesome aspect of the work so far. Our results indicate, however, that these patterns exist and deserve attention.

We have refrained from providing additional refinements, such as geological regionalisations or to assign probabilities to the forecasts as functions of time to the forthcoming event and its magnitude. The results of the present exploration indicate that such refinements are necessary and we hope that they would improve our success ratios, which are surprisingly high despite the simplicity of the criteria we have used.

This research was performed as part of Project III of the US-USSR cooperative programme in earthquake prediction. V.I. K.-B. was a Fairchild Scholar at the Seismological Laboratory, California Institute of Technology, and visiting professor at the Institute of Geophysics and Planetary Physics, University of California, Los Angeles. The following members of the Seismological Laboratory supported this research: C. Allen, D. Anderson, D. Harkrider, H. Kanamori, K. McNally, B. Minster. We also thank D. Eckelmann, T. Hearn, C. Johnson, J. Gardner, Y. Kagan, B. Bolt, J. Evernden, W. Lee, and R. Lamoreaux for comments and assistance. Financial support of the Office of Earthquake Studies, US Geological Survey is also acknowledged. This is publication no. 1882 of the Institute of Geophysics and Planetary Physics, University of California, Los Angeles and contribution no. 3261 of the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena.

Received 15 December 1978; accepted 8 November 1979.

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Regulation of acetylcholinesterase appearance at neuromuscular junctions *in vitro*

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The appearance of acetylcholinesterase (AChE) at newly formed nerve-muscle synapses depends on synaptic transmission. Synapses form when cultures are grown in the presence of acetylcholine receptor antagonists, but AChE does not accumulate at these synapses. The important component of transmission seems to be muscle activity. Treatment with dibutyryl cyclic GMP mimics muscle activity, directly inducing synaptic AChE appearance.

ACETYLCHOLINE receptors (AChRs) and acetylcholinesterase (AChE) are concentrated at adult motor endplates. The AChR is an integral membrane protein (see ref. 1 for review) restricted to the upper one-third of the postjunctional folds, whereas AChE is a peripheral protein associated with the basal lamina² throughout the primary and secondary synaptic clefts³. Even though AChRs and AChE are ultimately located at different sites, both molecules begin to accumulate within 24 h of nerve-muscle synapse formation *in vitro*⁴⁻⁶ and are present in approximately equal numbers at mature junctions^{3,7,8}. Therefore, one might suppose that the early AChR and AChE appearance at endplates is regulated in a coordinated way. Certain observations suggest, however, that synaptic AChRs and AChE are influenced quite differently by chronic blockade of synaptic transmission. As AChRs cluster at nerve-muscle contacts when spinal cord-muscle co-cultures are grown in the presence of α -bungarotoxin (α -BTX)⁹, this process is independent of synaptic transmission. On the other hand, discrete foci of AChE fail to appear when young chick embryos are injected repeatedly with α -neurotoxin or curare^{10,11}. These results have been taken as evidence that receptor antagonists block synapse formation. It is possible, however, that nerve-muscle synapses form in the presence of receptor antagonists (compare refs 12, 13) but that AChE does not accumulate in the absence of synaptic transmission. Data presented here indicate that this, in fact, is the case and, further, that activity of innervated muscle fibres is a crucial parameter regulating the early appearance of AChE at sites of transmitter release. The accumulation of AChRs and of AChE during neuromuscular junction formation is apparently not regulated in exactly the same way. A preliminary account of some of these experiments has been given elsewhere¹⁴.

As AChE accumulates rapidly at newly formed chick nerve-muscle synapses *in vitro*⁶, we used this culture system to examine in more detail the influence of synaptic transmission and muscle activity on AChE. Nerve processes emerge from spinal cord explants 10 h after the fragments attach to the culture surface, and synapses form soon after nerve-muscle contact. We have shown previously that AChE can be detected histochemically at some physiologically identified sites of transmitter release within 24 h of the onset of synaptic transmission and that after 4 d of co-culture, AChE reaction product is present at the majority of functional contacts⁶. Synaptic AChE activity was also detected by measuring the time course of synaptic currents recorded with an extracellular electrode. At adult endplates, AChE serves to terminate transmitter action, and this effect is reflected in the rate of synaptic current decay¹⁵⁻¹⁷. Based on the mean time constant of synaptic current decay ($\bar{\tau}_{\text{syn}}$) recorded at 30 °C, synapses formed in culture were arbitrarily divided into

three categories: fast ($\bar{\tau}_{\text{syn}} \leq 1.8$ ms), intermediate ($1.8 \text{ ms} < \bar{\tau}_{\text{syn}} < 2.6$ ms) and slow ($\bar{\tau}_{\text{syn}} \geq 2.6$ ms). Whereas 74% of the fast synapses stain for AChE, none of the slow ones stain.

Curare blocks appearance of synaptic AChE

Chick spinal cord explant-muscle co-cultures were prepared as previously described^{5,6}. Thin slices cut from the brachial segments of 14-d embryonic cords were added to established muscle cultures. Curare at a final concentration of 50 μM was added to some cultures for 3-4 d beginning at the time of explant addition. Innervated myotubes in control medium twitched vigorously and were continuously active. At 50 μM , curare blocked all synaptic activity and only a few isolated contractions were observed. This high concentration of curare did not block

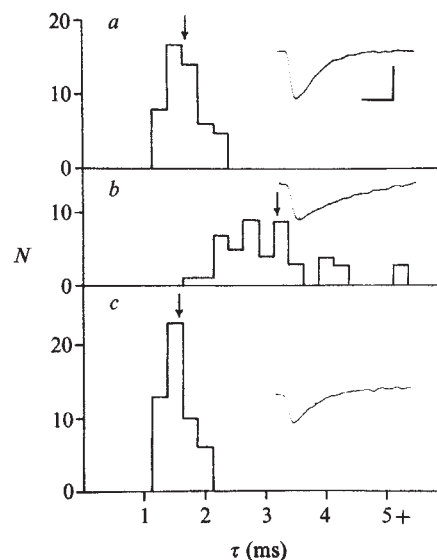


Fig. 1 Histograms of synaptic current decay time constants (τ_{syn}) recorded at 30 °C. Representative synaptic currents recorded with an extracellular microelectrode are shown as insets. Time constants were determined from exponential curves fitted to each synaptic current decay phase with an on-line computer. Arrows above each histogram indicate arithmetic means ($\bar{\tau}_{\text{syn}}$). At the control synapse (a), $\bar{\tau}_{\text{syn}}$ is < 1.8 ms, whereas at the synapse formed and maintained in the presence of curare (50 μM for 4 d; b), $\bar{\tau}_{\text{syn}}$ is > 2.6 ms. Both $\bar{\tau}_{\text{syn}}$ and the variance of τ_{syn} are increased at the curare-treated synapse, reflecting the lack of synaptic AChE activity^{6,17}. After data in b were obtained, the culture was placed in control medium without curare and returned to the incubator. Two days later, c was obtained by recording at another synapse in the same culture. $\bar{\tau}_{\text{syn}}$ and the variance of τ_{syn} were then comparable to control. Calibration bars, 2 ms and 200 μV .

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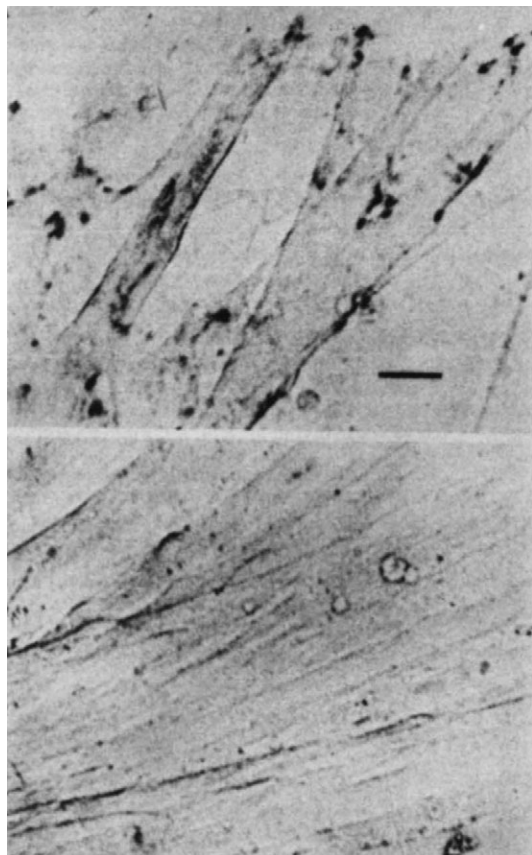


Fig. 2 Bright field view of control (upper) and 50 μ M curare-treated (lower) myotubes stained for AChE according to the method of Karnovsky and Roots³² 4 d after addition of spinal cord explants. Both fields were near (within 300 μ m of) the explants. Many patches of reaction product near fine nerve processes (not visible under bright field illumination) are evident on control myotubes. Few, if any, patches are present on curare-treated myotubes. Bar, 100 μ m.

the formation of synapses. Spontaneous synaptic activity was detected in most myotubes within 1–2 min of curare washout. In addition, autoradiographs of cultures treated with 125 I α -BTX immediately after curare washout showed that AChRs had clustered at sites of transmitter release.

Although synapses formed in the presence of curare, extracellularly recorded synaptic currents decayed relatively slowly (Fig. 1), indicating that AChE did not accumulate at sites of transmitter release. Judging from histograms of τ_{syn} constructed at 97 control and 89 curare-grown synapses (immediately after curare washout), the percentage of fast synapses decreased fivefold, whereas the percentage of intermediate synapses increased twofold and that of slow synapses threefold (Table 1). In the intermediate group, there was a shift towards higher values ($\bar{\tau}_{\text{syn}} > 2.2$ ms). The effect of curare was reversible; 1–2 d after cultures were returned to control medium, synaptic currents at most synapses decayed rapidly (Fig. 1).

Prolonged synaptic currents following curare washout were not due to an increase in the mean ACh channel open time. Estimates based on spectra of ACh-induced current fluctuations at control and curare-grown synapses were not significantly different (Table 1).

Histochemical experiments confirmed electrophysiological findings. The number of patches of AChE reaction product on myotubes near spinal cord explants in curare-grown cultures was reduced more than 10-fold compared with control (Fig. 2, Table 1). It cannot be assumed that all AChE patches are located at synapses, so sites of transmitter release were identified by extracellular recording, and then the same sites were relocated after fixation and histochemistry. Reaction

product was evident at 60 of 94 control synapses, but at only 1 of 49 synapses that had formed in the presence of curare. The reversibility of the curare inhibition illustrated above was also confirmed histochemically. Many AChE patches were evident 1–2 d after return to control medium.

Curare at 50 μ M did not affect the outgrowth of neurites, but in some cultures the nerve processes were more heavily stained for AChE than in control cultures. Chronic treatment with 2×10^{-8} M α -BTX also eliminated patches of AChE at synapses on innervated myotubes, but did not increase the extent of neuronal staining.

Curare blocks the appearance of 19.5S AChE

Several forms of AChE can be detected by sucrose density gradient centrifugation of extracts prepared from adult muscle (see ref. 18 for review). A high-molecular weight form sedimenting at 16S in rat¹⁹ and 19.5S in chick²⁰ extracts is present in innervated but not in denervated muscle. With our method of extraction (see Fig. 3 legend), we have never detected 19.5S AChE in chick muscle cultures without added neurones or in spinal cord cultures without target myotubes, nor have we reliably detected 19.5S AChE in spinal cord explant-muscle co-cultures; the number of synapses formed may be too small. However, as shown in Fig. 3a, a 19.5S peak was evident in muscle cultures seeded with a large number of dissociated spinal cord cells (see ref. 21); two other peaks of AChE sedimented at 7S and 11S. In different experiments, the 19.5S peak comprised 5–10% of the total activity. In five experiments, chronic exposure to curare decreased activity in the 19.5S peak at least fourfold (Fig. 3b) without decreasing the total AChE-specific activity (control, 0.314 ± 0.31 μ M per min per mg; curare, 0.395 ± 0.5 μ M per min per mg). The heavy form of AChE is restricted to the endplate region of innervated rat muscle¹⁹, but it is found in endplate-free regions of innervated chick muscle²⁰. Therefore, although a useful marker for the 'innervated state', it is not clear if the 19.5S form accounts for the AChE activity at synapses detected histochemically and electrophysiologically in our cultures.

The appearance of AChE at synapses depends on muscle activity

The effect of curare and α -BTX implies that something in the sequence of events that follow ACh release—ACh binding, ACh-induced increase in membrane conductance, muscle excitation and muscle contraction—is required for the accumulation of synaptic AChE. ACh binding is probably not sufficient; synaptic AChE was drastically reduced by chronic exposure to 10 μ M proadifen-HCl (SKF 525A). At this concentration, this local anaesthetic blocks ACh channels, but does not inhibit ACh binding²². Subthreshold synaptic conductance changes are probably not sufficient either; the appearance of AChE was substantially suppressed by tetrodotoxin, which blocks action potentials and, thus, impulse-mediated endplate potentials

Table 1 Comparison of control and curare-treated cultures

	$\bar{\tau}_{\text{syn}}$ (ms)			AChE patches (mean $\pm$ s.e.)	τ_0 (ms) (mean $\pm$ s.e.)
	1.0–1.8	1.8–2.6	> 2.6		
Control (97*)	59.8%	29.9%	10.3%	25.0 ± 2.0 (41†)	1.34 ± 0.08 (4‡)
Curare (89*)	12.4%	56.2%	31.5%	1.9 ± 0.3 (58†)	1.49 ± 0.16 (4‡)

Mean synaptic current decays ($\bar{\tau}_{\text{syn}}$) were determined at 30 °C as described in Fig. 1 legend. The number of patches of AChE reaction product (per spinal cord explant) was calculated by counting patches in the vicinity of neurites on undamaged myotubes. No more than 50 patches were counted on myotubes surrounding a single explant, so the control value is an underestimate. ACh channel open times (τ_0) were determined at fast synapses in control cultures and at slow synapses in curare-treated cultures using the technique described by Schuetz et al.³²

* No. of synapses at which $\bar{\tau}_{\text{syn}}$ was determined.

† No. of explants.

‡ No. of synapses at which τ_0 was determined.

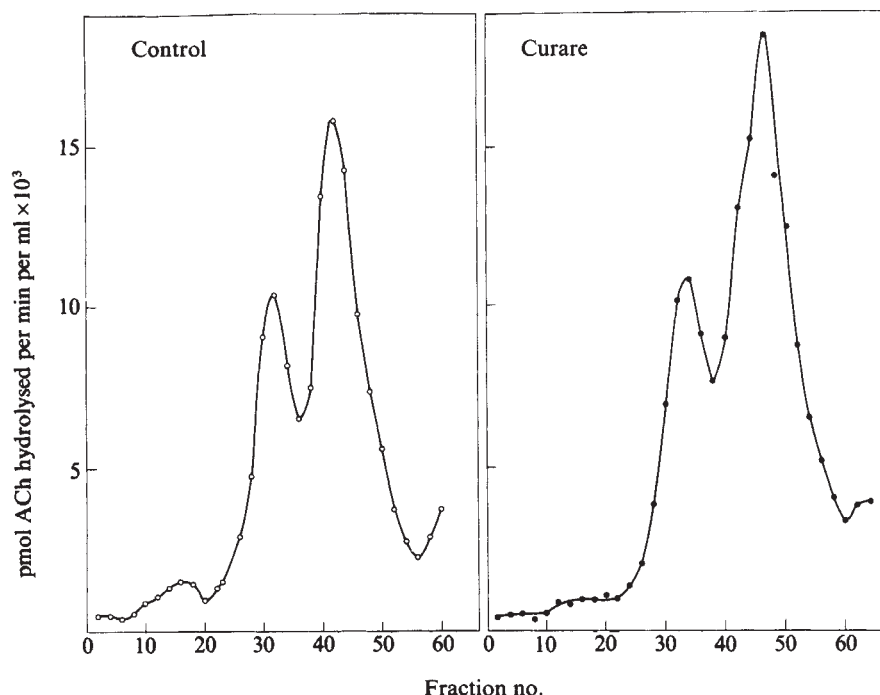


Fig. 3 Sucrose density gradient fractionation of different molecular forms of AChE. Chick muscles were plated at 3×10^5 cells per 60-mm culture dish. On day 3, 6-d embryonic chick spinal cords were dissociated and added ($2-3 \times 10^6$ cells per dish) to the muscle cultures in the presence of 10^{-5} M cytosine arabinoside with or without $50 \mu\text{M}$ curare. Five days later, three control and three curare-treated cultures were rinsed with Earle's balanced salt solution and the cells were collected by scraping with a Teflon policeman in phosphate-buffered saline containing 0.2 mM EDTA, pH 7.4. The cell suspension was centrifuged at 12,000g for 5 min and resuspended in 1 M NaCl, 0.5% Triton X-100, 0.05 M Tris-HCl, 0.2 mM EDTA and 1 mg ml $^{-1}$ DNase (Sigma), pH 7.3. After 1 h at 0 °C, this suspension was centrifuged at 12,000g for 15 min and the supernatant assayed for protein³⁴ and AChE activity³⁵. An aliquot (approximately 400–600 μg per 125 μl) was layered onto a 5–20% linear sucrose gradient and centrifuged in a Spinco SW41 rotor at 205,000g for 18 h at 4 °C. Sixty 0.25 ml fractions were collected and assayed for AChE by the radiometric method of Potter³⁶, as modified by Hall¹⁹. Three peaks of activity sedimenting at 7S, 11S and 19.5S were evident in the control gradient (left). Activity in the 19.5S peak was reduced at least fourfold by curare treatment (right). This figure shows the maximum 19.5S activity found in curare-treated cultures in five different experiments (platings). Gradient markers were alcohol dehydrogenase (4.8S), catalase (11.3S) and β -galactosidase (16S).

(e.p.p.s), but not impulse-independent miniature endplate potentials. Moreover, AChE did not accumulate in low concentrations ($3 \mu\text{M}$) of curare, which only reduced the mean e.p.p. by 50%.

Although $3 \mu\text{M}$ curare did not completely block e.p.p.s, most of the synaptic potentials were subthreshold; few innervated fibres were mechanically active. We therefore examined the role of muscle activity in the induction of synaptic AChE by electrically stimulating individual muscle fibres in the presence of curare. Spinal cord–muscle cultures were grown in $50 \mu\text{M}$ curare, and τ_{syn} was again determined at previously identified sites of transmitter release. Chronic stimulation in the presence of curare significantly decreased τ_{syn} (Fig. 4). Eight synapses at which τ_{syn} was greater than 2.21 ms were studied before and after stimulation (Table 2). Chronic stimulation in the presence of curare significantly decreased τ_{syn} at seven of these synapses to values typical of fast, AChE-positive sites. One other synapse was fast ($\tau_{\text{syn}} = 1.54$ ms) before stimulation, and its decay constant did not decrease further.

Influence of dibutylrlyl cyclic GMP on AChE

During these experiments, we found that low concentrations ($25-100 \mu\text{M}$) of dibutylrlyl cyclic GMP can reverse the curare inhibition of AChE accumulation. τ_{syn} was determined at 114 synapses in cultures grown in $50 \mu\text{M}$ curare with dibutylrlyl cyclic GMP: 64% were fast and only 3.5% were slow. These percentages are comparable to those obtained with untreated control

cultures (Table 1). The effect of dibutylrlyl cyclic GMP was also evident when AChE was assayed histochemically. Reaction product was present at 70% of identified synapses in cultures of curare plus dibutylrlyl cyclic GMP. Finally, dibutylrlyl cyclic GMP reversed the curare inhibition of 19.5S AChE assembly (Fig. 5) without increasing the total AChE activity. In six experiments, the ratio of total AChE (control/dibutylrlyl cyclic GMP treated) was 1.15 ± 0.25 (mean $\pm$ s.d.). Dibutylrlyl cyclic GMP did not 'induce' 19.5S AChE in muscle cells or in spinal cord neurones grown alone. Other agents, including GMP, GTP and dibutylrlyl cyclic AMP, did not reverse the curare inhibition of synaptic AChE accumulation or assembly of the 19.5S form.

Conclusions

The early accumulation of AChE at newly formed nerve–muscle synapses *in vitro* depends on synaptic transmission. By our physiological and histochemical criteria, the activity of the enzyme is greatly reduced at synapses that form in the presence of curare. Although 19.5S AChE is not restricted to adult chick endplates, it is significant that this form of the enzyme was selectively decreased in curare-blocked cultures.

Of the several events that follow synaptic transmission, muscle activity (electrical and/or mechanical) seems to be the crucial factor, as AChE does accumulate at curare-blocked synapses when innervated myotubes are stimulated directly. Lømo and Slater²³ have also emphasised the role of muscle activity in AChE accumulation at ectopic synapses on adult rat muscle fibres. Weinberg and Hall²⁴ suggested muscle activity as an explanation for their observation that 16S AChE (and AChE histochemical reaction product) reappears at denervated rat endplates when the denervated muscles are reinnervated at another site. Koenig and Vigny²¹ detected 16S AChE in control rat spinal cord–muscle co-cultures but not in inactive cultures grown in the presence of tetrodotoxin²⁵. Inadequate muscle activity may explain why no evidence was obtained for AChE activity at nerve–muscle synapses when chick muscle cultures were seeded with a relatively small number of dissociated spinal cord neurones²⁶. In this system, innervated myotubes twitch more frequently than uninnervated myotubes, but the overall activity is far less than that observed in innervated myotubes near intact spinal cord explants.

Table 2 Effect of direct muscle stimulation on τ_{syn}

Synapse no.	Before	After	% Change
1	2.21 ± 0.70	1.74 ± 0.32	21.3
2	3.09 ± 1.39	1.74 ± 0.39	43.7
3	2.25 ± 0.73	1.35 ± 0.20	40.0
4	3.06 ± 1.04	2.05 ± 0.71	33.0
5	3.19 ± 1.56	1.87 ± 0.49	41.4
6	3.14 ± 0.87	1.75 ± 0.37	44.3
7	2.59 ± 1.15	1.80 ± 0.41	30.5
8	2.42 ± 0.92	2.30 ± 0.65	5.0
9	1.54 ± 0.35	1.51 ± 0.21	1.9

τ_{syn} ($\pm$ s.d.) was determined from the time constants of decay of at least 20 extracellular potentials recorded at the same synapses in curare-treated cultures before and after direct electrical stimulation of myotubes in the presence of $50 \mu\text{M}$ curare for 8–12 h at 2–3 Hz and 35–36 °C.

In contrast to the situation with AChE, the clustering of AChRs in the postsynaptic membrane does not depend on activity. Thus, the formation of AChR clusters in the postsynaptic membrane and the accumulation of AChE in the synaptic cleft are not necessarily linked. This result does not rule out the possibility that AChE and AChRs are synthesised and transported to the cell surface in a coordinated manner. Muscle activity may simply ensure the anchorage of AChE to the basal lamina of the synaptic cleft.

Muscle activity is probably not the sole determinant of synaptic AChE. Nerve extracts slow the rate of AChE disappearance, measured biochemically, from adult muscle maintained in organ culture^{27,28}, and saline extracts of central nervous tissue increase total AChE of uninnervated embryonic myotubes in culture^{29,30}. Although it is difficult to compare these biochemical estimates of total (intracellular as well as extracellular) AChE with our physiological and histochemical assays, which detect AChE exposed on the cell surface at synapses, our emphasis on the importance of muscle activity is not inconsistent with the notion that synaptic AChE is also regulated by soluble factors supplied by motor nerves. The fact that AChE and AChRs are restricted to sites of transmitter release argues for a local neural influence. Moreover, we have identified AChE-positive and AChE-negative synapses within 500 μm of one another on the same myotube (Fig. 7 in Rubin *et al.*⁶). If both contacts were formed at approximately the same time and if both were exposed to the same degree of muscle activity, some additional factor must be required. Muscle activity may be 'permissive', allowing neuronal factors to exert their effects.

Addition of dibutyl cyclic GMP to cultures grown in curare mimics the effects of muscle activity on synaptic AChE and on assembly of the 19.5S form of the enzyme. Of interest in this regard is the finding that stimulation of adult skeletal muscle results in an increase in cyclic GMP levels³¹. Neither result demonstrates a necessary connection between activity and cyclic GMP in regulating the appearance of AChE. Perhaps a stronger argument for such a connection could be made if muscle activity and cyclic GMP treatment resulted in phosphorylation of the same muscle proteins.

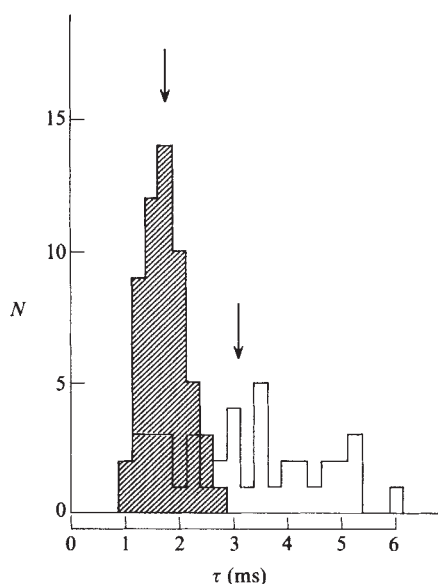


Fig. 4 Chronic muscle stimulation in the presence of curare decreases $\bar{\tau}_{\text{syn}}$. This culture was grown in 50 μM curare for 4 d, at which time synaptic current decays (recorded during a brief period of curare washout) at this synapse were prolonged (unshaded histogram). Immediately after the recordings were made, the culture was returned to medium with 50 μM curare, and the innervated myotube was electrically stimulated at 3 Hz for 11 h at 35–36°C. Immediately after stimulation and curare washout, the same synapse was relocated and a clear decrease in $\bar{\tau}_{\text{syn}}$ was observed (shaded histogram). The variance of τ_{syn} was also decreased. All recordings were obtained at 30°C. Arrows indicate $\bar{\tau}_{\text{syn}}$.

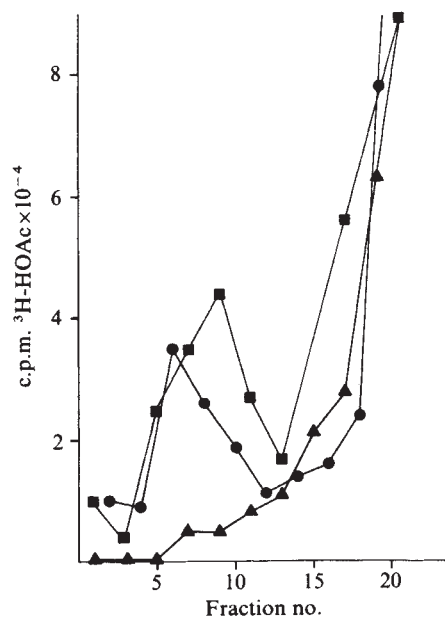


Fig. 5 19.5S AChE appears in spinal cord-muscle cell cultures grown in curare plus dibutyl cyclic GMP. Cultures were grown without added drugs (●), with 50 μM curare (▲) or with 50 μM curare plus 100 μM dibutyl cyclic GMP (■). Extracts were prepared and sucrose gradients run, as described in Fig. 3 legend. Only the high-molecular weight region of the gradient is shown. Assembly of 19.5S AChE was nearly completely blocked by curare, but this inhibition was reversed by adding dibutyl cyclic GMP.

This work was supported by USPHS grant NS 11160, by the Muscular Dystrophy Association of America and by a Helen Hay Whitney Foundation Postdoctoral Fellowship (L.L.R.), an NIH National Research Service Award T32GM07226 (S.M.S.) and a Muscular Dystrophy Association Postdoctoral Fellowship (C.L.W.).

Received 15 February; accepted 31 October 1979.

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Interactions between the carbohydrate chains of hyaluronate and chondroitin sulphate

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Hyaluronic acid-derivatised beads spontaneously agglutinate with chondroitin sulphate-derivatised beads although neither bead type shows any self-agglutination. The interaction between hyaluronate and chondroitin sulphate is specific for these two glycosaminoglycans and appears to occur between their carbohydrate chains.

ALMOST all mammalian cells contain glycosaminoglycans as a part of their coat and extracellular material¹. All of the glycosaminoglycans so far studied, except hyaluronic acid, are sulphated and covalently linked to protein^{1,2}. Hyaluronate, by contrast, is known to bind noncovalently to the protein portion of the proteoglycans³. The biological functions of these polymers are poorly understood. Glycosaminoglycans are apparently important structural molecules and have long been thought to function in the regulation of cation and H₂O binding as well as the control of macromolecular distribution by steric exclusion⁴. More recently, glycosaminoglycans have been implicated in cell adhesion^{1,5,6}, organogenesis⁷, and differentiation⁸. In particular, the excellent correlation between cell migration *in vivo*⁸⁻¹¹ and *in vitro*¹² and the elevated levels of hyaluronate and chondroitin sulphate suggest that a cell-glycosaminoglycan interaction may be important for cell movement. Some of these biological effects may be due to the ability of the glycosaminoglycans to bind to cell surface components.

Binding and chemical cross-linking studies¹³ have indicated that the cell-surface glycoprotein fibronectin exists in close proximity to both hyaluronic acid and the sulphated glycosaminoglycans. This report demonstrates that chondroitin sulphate and hyaluronic acid are capable of a specific binding interaction with each other and this interaction occurs between the carbohydrate chains of the polymers. This observation raises the possibility that glycosaminoglycans located at the cell surface may function as binding sites and, therefore, be involved in cellular adhesive and recognition phenomena.

Derivatisation of the glycosaminoglycans

Solutions of chondroitin-4-sulphate (C-4-S), chondroitin-6-sulphate (C-6-S), hyaluronic acid (HA), dermatan sulphate, and heparin were dialysed and protein levels were shown to be <0.1%. The polymers were attached to Dowex AG 50 W-X-1 beads (50–100 mesh) by first converting the beads to the sulphonamide form and then incubating them with one of the

Table 1 Binding of chondroitin sulphate and hyaluronic acid to polysulphonamide beads

Concentration of glycosaminoglycan (µg per 100 mg beads, wet wt)	Glycosaminoglycan attached (µg per mg beads, dry wt)	Concentration of glycosaminoglycan (µg per 100 mg beads, wet wt)	Glycosaminoglycan attached (µg per mg beads, dry wt)
Chondroitin 6-sulphate		Hyaluronic acid	
15,000	100–130	15,000	25–30
1,500	10–12	1,500	12–15
150	1.0–1.4	150	5–7
15	0.1	15	1–5
1.5	<0.04		

Chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate and heparin were purchased from Sigma Chemicals. Hyaluronic acid was a gift from Dr B. Toole. All the glycosaminoglycan solutions were dialysed for 24 h against distilled water, lyophilised and stored, desiccated, until use. To determine the degree of molecular weight homogeneity, the compounds were chromatographed on Sephadex G-200 in a buffer¹⁴ containing 1% SDS (w/v), 10⁻⁴ M Na₂EDTA, 10⁻³ M dithiothreitol, 10⁻³ M sodium azide and 0.05 M phosphate buffer (pH 7.2). Fractions were assayed for carbohydrate with phenolsulphuric acid¹⁵ and for protein by the Lowry method¹⁶, by UV absorbance at 260 and 280 nm (ref. 17), 215 and 225 nm (ref. 18) and by the Bio-Rad protein assay¹⁹. Hyaluronic acid eluted in the Sephadex void volume whereas the other glycosaminoglycans were retarded and eluted as a single, symmetrical peak when assayed for carbohydrate. In assay conditions that could detect 10 µg protein, no protein was detectable in aliquots containing 10 mg of either chondroitin sulphate or hyaluronic acid. These carbohydrate preparations, therefore, contained less than 0.1% protein. To derivatise beads with the glycosaminoglycans, Dowex AG 50 W-X-1 (50–100 mesh) beads (Bio-Rad) were converted to the sulphonamide form by incubation in ammonium hydroxide (5 g beads per 10 ml NH₄ OH) at room temperature for 24 h. The beads were then washed exhaustively in distilled water and 0.1 g wet weight of the beads was added to 1 ml of an aqueous solution of either chondroitin-6-sulphate (0.0015–15 mg ml⁻¹) or hyaluronic acid (0.015–15 mg ml⁻¹). Each incubation also contained five times more (w/w) of the catalyst 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (Sigma) than glycosaminoglycan. The glycosaminoglycan solution and beads were incubated for 48 h at 35–37 °C after which the beads were washed extensively in distilled water and, finally, in aseptic, phosphate-buffered saline (PBS) in which they were stored. Aliquots of the derivatised beads were analysed by subjecting the beads to hydrolysis in 90% formic acid at 100 °C for 6 h. The hydrolysates were evaporated to dryness and the resultant monosaccharides were assayed for uronic acid by the Dische assay²⁰ in the case of hyaluronic acid. To monitor the derivatisation of the chondroitin sulphates, tritiated chondroitin sulphates (1 × 10¹⁰ c.p.m. per mg) were prepared by galactose oxidase treatment followed by reduction with sodium borotritide as described previously¹². The labelled compound was then used in the carbodiimide-catalysed derivatisation procedure. The hydrolysed monosaccharides were evaporated to dryness, redissolved in H₂O and counted in a scintillation counter. Values represent the ranges of two separate experiments.

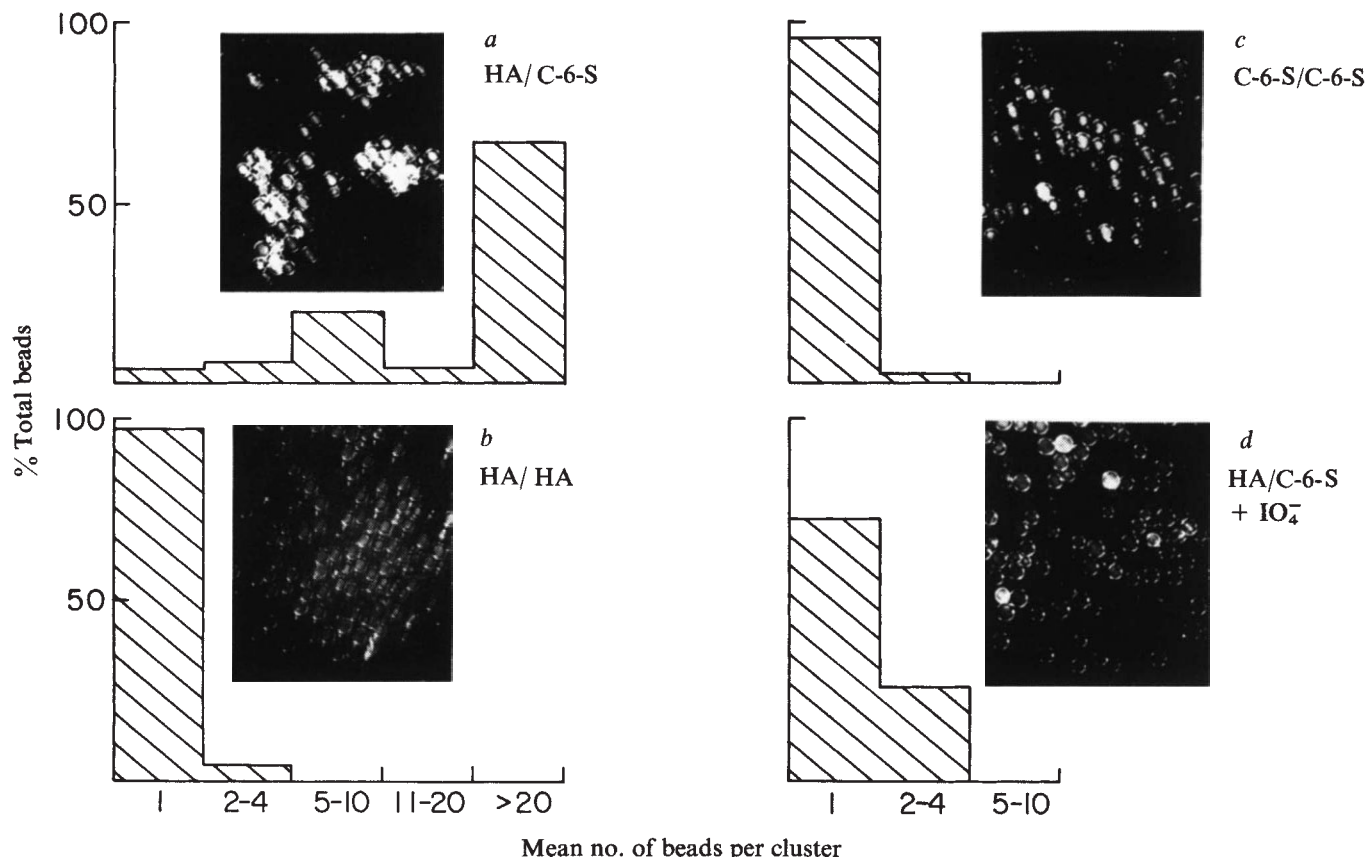


Fig. 1 The heterologous (a) and homologous (b, c) interaction of sulphonamide beads derivatised with either hyaluronic acid or chondroitin-6-sulphate. Agglutination assays were performed in 35 × 10 mm Falcon tissue culture dishes and initiated by adding 0.01 g of each of the desired bead types in 0.1 ml PBS (pH 7.4) to 2 ml of PBS at 4 °C. Approximately 30 µg of hyaluronic acid or 100 µg of chondroitin 6-sulphate were derivatised to each mg of beads (dry weight). The dishes were agitated gently for 5 min at 4 °C. Agglutination was scored 12 h later by counting beads occurring in clusters or as single beads under a dissecting microscope. Dishes were shaken before counting the beads to separate loosely associated beads. The number of beads in each of the initial 0.1 ml aliquots was determined before the experiments were begun and the data are expressed as the mean number of beads per cluster. A total of 300 beads were counted in each agglutination assay. The experiment was repeated 20 times with different bead preparations and the results were quantitatively identical.

polymer solutions in the presence of a carbodiimide catalyst¹². Details of the derivatisation and hydrolysis procedures are given in the legend to Table 1. The data in Table 1 show that the amount of HA and C-6-S derivatised to the beads is proportional to the amount of each compound present in solution during the incubation with carbodiimide. When HA and C-6-S were each present at 15 mg ml⁻¹, the highest concentration tested during the derivatisation incubation, more than 100 µg C-6-S became attached to 1 mg dry weight of the beads, whereas 25–30 µg hyaluronic acid became attached in identical conditions.

Agglutination assays with derivatised beads

Agglutination assays were performed as described in the legend to Fig. 1. Agglutination is pronounced when C-6-S beads are mixed with HA beads. Neither bead type shows any tendency towards self-agglutination. When HA beads are mixed with C-4-S beads, there is an agglutination reaction although it is approximately 40% as extensive as the reaction that occurs when HA beads and C-6-S beads are mixed. HA beads show only minimal agglutination when mixed with beads derivatised with heparin or dermatan sulphate. It is not clear whether the HA/CS interaction is specific for C-6-S and if this compound is contaminating our preparations of C-4-S, or whether hyaluronate will interact with either of the two chondroitins but with C-4-S to a lesser degree. The agglutination reactions described here were unaffected by temperature from 4° to 37 °C, calcium ion concentration of 0–20 mM, osmolarity of 0–300 mosM, and time of 5 min to 12 h.

Since the amount of sulphated glycosaminoglycan, other than C-6-S, bound to the beads was not measured, the decreased degree of agglutination may reflect a difference in concentration. There is no measurable agglutination among HA beads themselves or among beads subjected to the derivatisation procedures but without glycosaminoglycans. Hyaluronic acid beads initially agglutinate weakly with the underivatized beads but the interaction disperses within several hours.

C-6-S beads also show no self-agglutination, and they do not agglutinate underivatized beads or beads coated with other sulphated glycosaminoglycans. The absence of such homotypic agglutination can be seen in Fig. 1b and c. By contrast, the agglutination that occurs when these two bead types are mixed is shown in Fig. 1a. It is unlikely that the agglutination between the HA beads and the C-6-S beads reflects a nonspecific ionic attraction of charged polymers since neither bead type shows any tendency to self-agglutinate in the conditions tested here.

Since the glycosaminoglycans used in these experiments are 99.9% free of protein it is probable that the agglutination results from interactions between the carbohydrate chains of the hyaluronic acid and chondroitin sulphate. Further evidence for this conclusion can be seen in Fig. 2. The data in the left column of Fig. 2 show that the HA bead/C-6-S bead interaction is sensitive to mild periodate oxidation of either bead type. However, periodate oxidation of C-6-S has less effect on the HA/C-6-S interaction than oxidation of the hyaluronic acid polymer. This may reflect the relative resistance of C-6-S to periodate oxidation¹⁹. The right-hand column shows that HA bead/C-4-S bead interaction is abolished when C-4-S beads are treated with galactose oxidase. The lower right panel of Fig. 2

shows that the inhibition of agglutination by galactose oxidase can be partly reversed if the oxidised C-4-S beads are subsequently reduced with borohydride. C-4-S beads treated with galactose oxidase and 10–100 mM of the competitive substrate, galactose, retained their ability to agglutinate with HA beads. Heat-denatured galactose oxidase had no effect on the HA bead/C-4-S interaction and the active enzyme had no effect on HA beads. These results are consistent with the specificity of galactose oxidase for the C-6 hydroxyl group of galactosides and acetylated galactosaminides. Beads derivatised with oligosaccharide fragments of HA or C-6-S, prepared by digestion with testicular hyaluronidase and estimated to contain 8 residues by gel chromatography, agglutinated to a lesser extent than the beads derivatised with untreated polymers. Trypsin had no effect on HA bead/C-6-S bead interaction. In another experimental series, hyaluronate and chondroitin sulphate were subjected to mild base hydrolysis and reduction by sodium borohydride²² to release residual protein. When these polymers were attached to beads, the HA bead/C-6-S bead agglutination occurred normally. These data, coupled with the relative absence of protein in the glycosaminoglycan preparations, suggest strongly that agglutination between beads containing hyaluronic acid and chondroitin sulphate represents a carbohydrate-carbohydrate interaction.

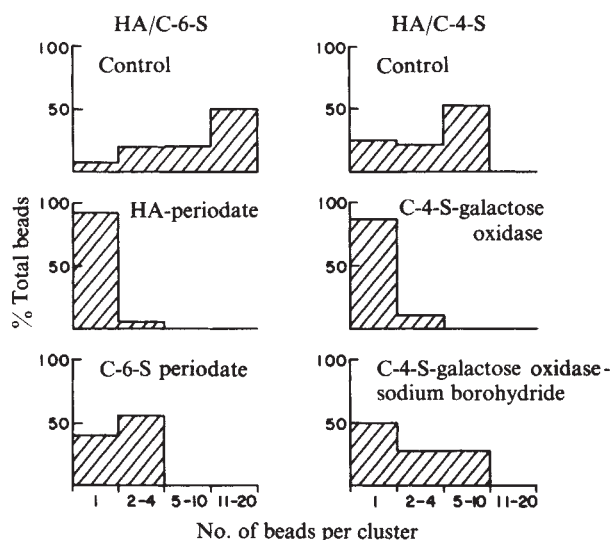


Fig. 2 The effects of periodate and galactose oxidase on the agglutination of HA and CS beads. Beads were derivatised with either hyaluronic acid (30 µg per mg beads) or C-6-S (100 µg per mg beads) as described in Table 1. One ml of an aqueous solution containing 10–1,000 molar excess of periodate (based on molecular weight of 1×10^6 for hyaluronic acid and 4×10^4 for C-4-S) was added to 100 mg beads (wet weight). The beads were incubated at room temperature for 20–60 min. Periodate oxidation reaction was followed by recording the decrease in absorbance at 223 nm (ref. 21). Ten mg beads (wet weight) derivatised with C-4-S were incubated with 50 units of galactose oxidase (*Polyporus circinatus*, Sigma, 100 U per mg protein) in sterile PBS (pH 7.4) for 6 h at 37 °C after which an additional 10 units were added and incubated for another 12 h. The enzyme solution was decanted and beads were washed in sterile PBS twice and heated at 100 °C for 15 min to inactivate the residual enzyme. One half of the enzyme-treated beads were subsequently incubated with 10 mM sodium borohydride for 10 h at room temperature. Unreacted sodium borohydride was then destroyed by careful addition of 0.1 M acetic acid. Oxidised and reduced C-4-S beads were again washed several times and then assayed for their ability to agglutinate with HA-beads as described in Fig. 1. Incubation of C-4-S beads with galactose oxidase and the competitive substrate, galactose, with boiled enzyme or with sodium borohydride, glycerol or acetic acid did not alter agglutination with HA beads. This experiment, like those reported in Figs 3 and 4, was repeated four times, with different bead preparations and the results was quantitatively identical. A total of 300 beads were counted for each assay.

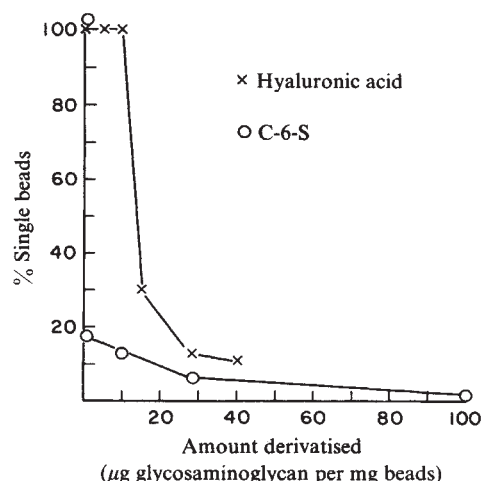


Fig. 3 The agglutination of HA beads with C-6-S beads as a function of the amount of polymer derivatised to beads. Beads were incubated with 0.1 to 15 mg per 100 mg beads of either HA or C-6-S together with 5 times (w/w) that amount of carbodiimide as described in Table 1. Aliquots of beads derivatised with 0–100 µg C-6-S per mg bead (dry weight) were mixed with HA beads (30 µg HA per mg bead, dry weight). Similarly, aliquots of beads derivatised with 0–30 µg HA per mg bead were mixed with C-6-S beads (100 µg C-6-S per mg bead). Beads were shaken gently for 5 min and then counted as described for the agglutination assay in Fig. 1. Agglutination was expressed as per cent single beads. A total of 300 beads were counted for each assay.

The agglutination between HA beads and C-6-S beads is dependent on the amount of each polymer bound to the beads. In Fig. 3, agglutination is determined as a function of the amount of HA and C-6-S derivatised to the beads. C-6-S beads with as little as 0.1 µg polymer per mg (dry weight) bead are able to agglutinate maximally with HA beads containing 30 µg HA per mg bead. Hyaluronic acid beads, however, require at least 30 µg HA per mg bead to agglutinate maximally with beads containing 100 µg C-6-S per mg beads.

The agglutination of the C-6-S beads with the HA beads is sensitive to either hyaluronic acid or C-6-S in solution. The standard agglutination reaction was performed with increasing amounts of soluble glycosaminoglycans added to the incubation mixture (Fig. 4). The agglutination appears to be more sensitive to hyaluronic acid since as little as $5 \mu\text{g ml}^{-1}$ of this polymer is able to inhibit the agglutination of the two bead types. C-6-S in solution is not an effective inhibitor below concentrations of about $50 \mu\text{g ml}^{-1}$. After the addition of C-6-S or hyaluronic acid, the bead clusters begin to disperse so that within 1–2 h beads are no longer agglutinated. Neither oligosaccharide fragments nor monosaccharides of hyaluronic acid or C-6-S were able to inhibit bead agglutination.

Discussion

Beads derivatised with hyaluronic acid spontaneously agglutinate beads derivatised with C-6-S when the two bead types are mixed in PBS or in distilled H_2O . The agglutination occurs within minutes at temperatures between 4 °C and 37 °C and remains stable for as long as one day. Hyaluronic acid beads also interact with beads coated with other sulphated glycosaminoglycans such as C-4-S, heparin and dermatan sulphate although to a lesser extent. Homologous interactions among beads coated with one glycosaminoglycan do not occur.

The interactions described here were obtained using glycosaminoglycan preparations containing less than 0.1% protein and this fact alone suggests that the agglutination reflects an interactive specificity between the carbohydrate chains of hyaluronic acid and chondroitin sulphate. This conclusion is further supported by other observations. The HA/sulphated

glycosaminoglycan interaction is sensitive to mild periodate oxidation, to treatment with testicular hyaluronidase, which hydrolyses the carbohydrate chain of the polymers to oligosaccharides, and to galactose oxidase treatment of C-4-S. The interaction was not disrupted by trypsin treatment nor by mild base hydrolysis of the polymers in conditions reported to release protein²². Although periodate can affect protein, the conditions used here minimise protein oxidation²³. The galactose oxidase used in this study was not a homogeneous enzyme and the preparation may have contained other degradative enzymes that inhibited the agglutination between the HA beads and the C-4-S beads. Three experiments suggest, however, that the inhibition was due to the action of galactose oxidase and not to impurities. First, the enzyme preparation had no effect on HA-beads. Second, the inhibition of agglutination that resulted when C-4-S beads were treated with galactose oxidase could be partly overcome by subsequent treatment with sodium borohydride. Third, galactose oxidase in the presence of the competitive substrate, galactose, had no effect on C-4-S beads. These results indicate that the galactose oxidase preparation was interacting with C-4-S polymer in a manner consistent with its catalytic specificity. It seems very unlikely, therefore, that the agglutination described in this report is mediated by protein. The most reasonable explanation is that the agglutination reflects an interaction between the carbohydrate chains of chondroitin sulphate and hyaluronic acid.

In plants and bacteria, the binding between certain homologous and heterologous carbohydrate chains is known to occur. Cellulose, the most abundant biological molecule, consists of lengthwise aggregates of glucose polymers. Also, galactomannans, glucomannans, arabinoxylans and celluloses can bind to agarose and K-carageenans. The plant pathogen *Xanthomonas* may bind to host cellulose via its galactomannan chains³. Suggested mechanisms for such binding between carbohydrate chains include aggregated helices, interactions along specific regions to form 'egg-box' structures, or combinations of the two³.

The mechanism of binding between the hyaluronate and sulphated glycosaminoglycan chains described here is entirely

unknown. Homologous chains of glycosaminoglycans are known to aggregate²⁵ and to form helices in solution²⁶⁻²⁸. The homologous interaction seems to differ from the HA bead/CS bead agglutination described here since homologous beads did not agglutinate with one another. The ability of soluble polymers to inhibit and to reverse the agglutination of the derivatised beads suggests that the binding may be an equilibrium with an appreciable dissociation rate.

There is considerable evidence to show that hyaluronate can interact with proteoglycan. Muir and her colleagues have shown, for example²⁹, that the elution profile of disaggregated, cartilage proteoglycans on Sepharose 2B is changed markedly by the addition of as little as 0.625% hyaluronate. The hyaluronate and the proteoglycan elute together earlier than either would have alone. It is not proposed here that the carbohydrate-carbohydrate interaction is the mechanism for such observations—merely that it is another mechanism for specific interactions among proteoglycan components.

There are at least two reasons that could account for the failure of previous experiments to detect a heterologous interaction between the carbohydrate chains of HA and CS. First, no obvious precipitation occurs when the soluble compounds are mixed. This may indicate that the number of saccharide chains involved in complex formation in solution is small. Second, when hyaluronate/proteoglycan interactions are under examination, there is no reason to believe that the heterologous interaction between the carbohydrate chains would interfere with the hyaluronate/protein interaction. The avidity with which hyaluronate binds to CS may, in fact, be much less than that with which it binds to the protein of the proteoglycans. These comparisons will require further experimentation for the precise determinations of true binding constants.

Much research has implicated the glycosaminoglycans, particularly hyaluronate and chondroitin sulphate, in cell-cell adhesion^{1,5}, cell-substrate adhesion^{6,12}, organogenesis⁷, and differentiation⁸. In many embryos, the control of deposition, degradation, and the spatial arrangements of the glycosaminoglycans suggest that they may have a key role in morphogenesis. Precisely how these polymers can effect adhesive behaviour and morphogenetic phenomena is not known but a specific binding between heterologous, carbohydrate chains could represent one mechanism.

This work was supported by a postdoctoral fellowship to E.A.T. from the NCI of Canada, and by a research career development award (National Institute of Child Health and Human Development) and a research grant (NCI) to S.R.

Received 23 July; accepted 31 October 1979.

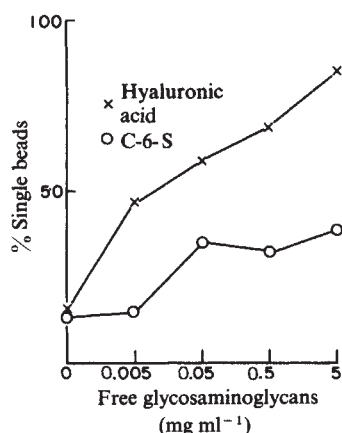


Fig. 4 The effect of soluble glycosaminoglycans on the agglutination of HA and C-6-S beads. Solutions of glycosaminoglycans were prepared by dissolving from 5 μ g to 5 mg of either HA or C-6-S polymer and adding the solutions to agglutinated C-6-S and HA beads. Beads and the glycosaminoglycan solutions were incubated at 4 °C for 10 h. Beads were then counted and the extent of agglutination expressed as per cent single beads. Solutions containing monosaccharide or oligosaccharide fragments (approximately 4–8 residues) had no effect on the interaction between HA and C-6-S beads. Oligosaccharide fragments were prepared by digestion with testicular hyaluronidase (Worthington 0.4 mg ml⁻¹) in 0.1 M sodium phosphate buffer containing 0.15 M sodium chloride at pH 5.3²⁴. The molecular weight was estimated by chromatography on Sephadex G-200 column in SDS buffer. Sephadex fractions were assayed for carbohydrate with phenol sulphuric acid¹⁵.

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LETTERS

Distribution of polarised radio emission in M31

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Observations yielding the distribution of large-scale magnetic fields in nearby spiral galaxies are still extremely scarce. Optical polarisation of starlight has been observed in the disks of M31, M51, M81 and some other spiral galaxies¹⁻³, but these measurements are too few to reveal the general distribution and direction of the magnetic fields. Segalovitz *et al.*⁴ observed the distribution of the polarised radio emission in M51 at $\lambda 21$ cm and $\lambda 6$ cm with sufficient linear resolution (2.6×3.6 kpc) and sensitivity to conclude that the magnetic field in M51 is predominantly directed along the spiral arms. The first results of detailed polarisation measurements of the emission of M31 at $\lambda 11$ cm (ref. 5) indicated that in the southern part of the bright ring of radio emission the magnetic field is generally aligned along the ring. We show here that this early conclusion seems to hold for the whole of the ring.

The observations were carried out with the 100-m telescope of the Max-Planck-Institut für Radioastronomie at Effelsberg between 1977 and 1978. The dual-channel correlation polarimeter in the secondary focus operated at 2,700 MHz (system temperature, 100 K; bandwidth, 80 MHz; observed half-power beamwidth, 4.4 arc min). The flux densities and polarisation characteristics of the calibration sources 3C48, 3C138 and 3C147 were taken from Baars *et al.*⁶ and Wardle and Kronberg⁷.

The ratio of main beam brightness temperature T_b (K) to the flux density S (Jy) was found to be 2.3 ± 0.1 . 3C147 was used to determine the spurious instrumental polarisation, which was found to be highest at 2 arc min distance from the source with $\sim 0.5\%$ of the total emission.

Figure 1 shows the $\lambda 11.1$ cm polarisation map of M31 superimposed on an optical picture. The map covers the area $-81 < \lambda_A < +81$ arc min, $-45 < \beta_A < +45$ arc min, where the coordinates λ_A and β_A lie along the major and minor axis, respectively. The area was observed in three parts, each overlapping by 10 arc min in λ_A . The northern part and the central part were scanned both in λ_A and β_A (~ 10 times each), while the southern part was scanned only in β_A (~ 20 times). For the northern and the central parts the averages of the maps in λ_A and of the maps in β_A were combined using a technique for optimising the base levels developed by C. J. Salter. By applying this technique to the maps in Stokes parameters Q and U separately, any polarised foreground emission varying linearly across the map is eliminated. (Faraday rotation in the foreground is not affected by this procedure.)

The polarisation temperatures derived from $(Q^2 + U^2)^{1/2}$ suffer from a systematic positive bias due to noise. All values in Fig. 1 have, therefore, been corrected as proposed by Wardle and Kronberg⁷. The r.m.s. noise σ in the final map is 3 mK in main beam polarisation brightness temperature T_b^p .

A map of the total intensity of the area at $\lambda 11.1$ cm was obtained from the total power channels of the same observations (Fig. 2); the r.m.s. noise is 5 mK in main beam brightness temperature T_b . This new total power map does not differ significantly from the map published by Berkhuijsen and Wielebinski⁸, but it has a slightly better angular resolution and lower r.m.s. noise. The new map extends further in β_A so that the

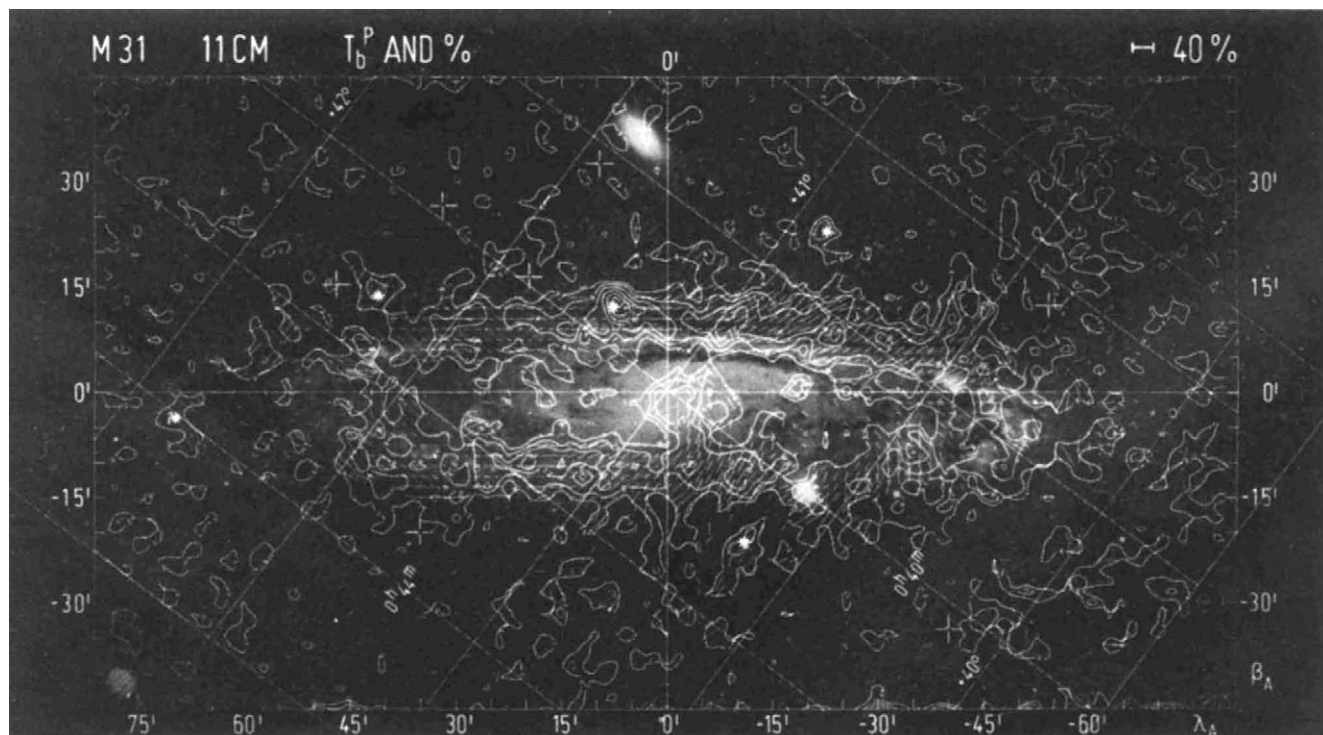


Fig. 1 Contour map of main beam polarisation brightness temperature T_b^p at $\lambda 11.1$ cm superimposed on an optical picture of M31 (photograph from Hale Observatories). The first contour level is 4 mK ($= 1.3\sigma$) in T_b^p ; the contour interval is 4 mK. The length of the vectors is proportional to the percentage polarisation p which was only computed if $T_b^p > 3$ mK and $T_b > 15$ mK. Asterisks show the position of polarised point sources 5C3.73, 107, 120, 126 and 163.

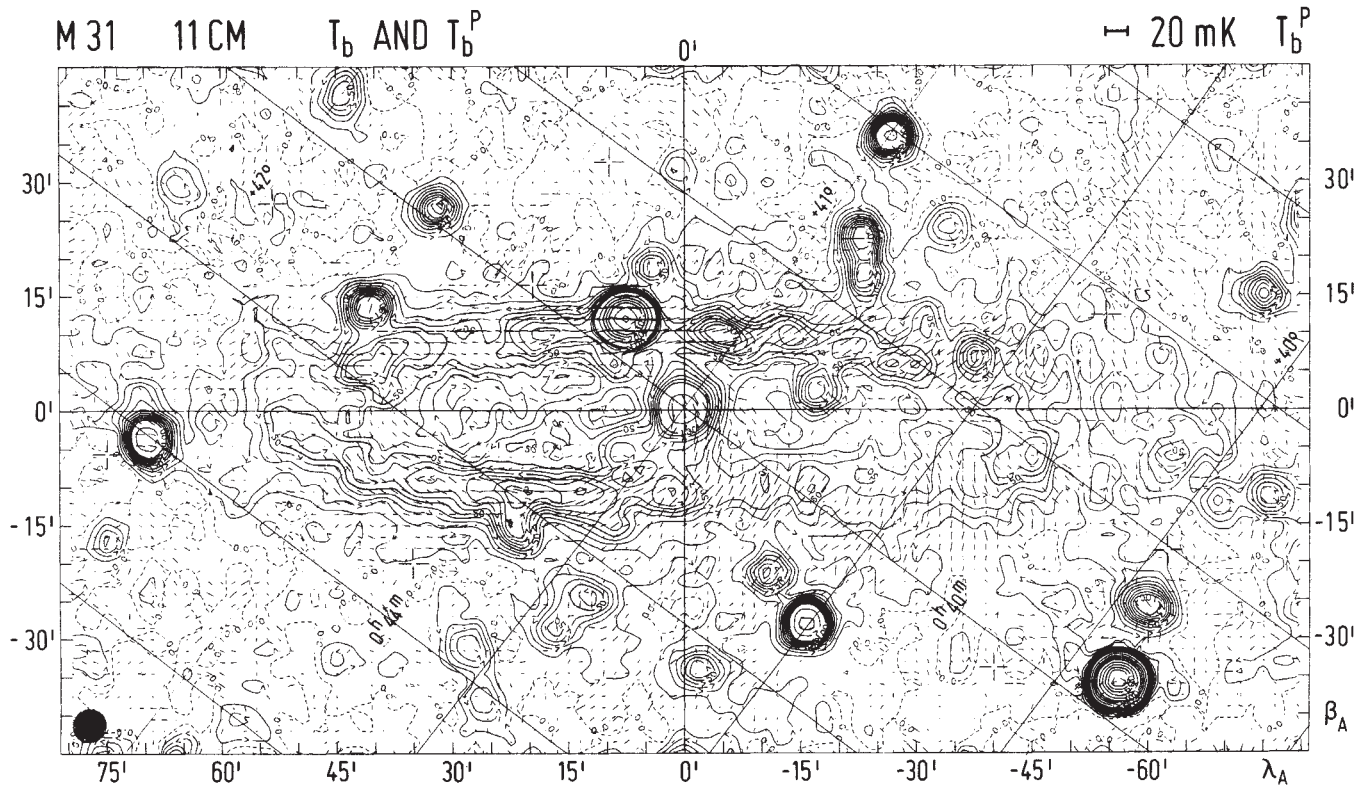


Fig. 2 Contour map of the main beam brightness temperature T_b . Contour levels are: 0, 10, ..., 100, 150, ..., 300, 400, ..., 800 mK. The length of the vectors is proportional to the main beam polarisation brightness temperature T_b^p .

zero level could be determined with more confidence. The zero level in Fig. 2 is higher by 5 ± 3 mK compared with the old map.

The total power map of Fig. 2 was used to compute the degrees of polarisation as shown by the vectors in Fig. 1.

Figure 1 contains the optically brightest parts of M31 out to a radius $R = 16$ kpc along the major axis adopting a distance to M31 of 690 kpc⁹. The resolution of 4.4 arc min corresponds to 0.9×3.2 kpc in the plane of M31 (inclination $i = 74^\circ$; $i = 0^\circ$ is face-on). This is the finest linear resolution used to date for observing the distribution of the polarised radio emission in a nearby spiral galaxy. Because the width of the arms on the major axis is ~ 1.5 kpc (ref. 10) this resolution is small enough to study the large-scale magnetic field in some detail.

The outstanding feature of Fig. 1 is the concentration of the polarised emission in a ring around the centre. This ring approximately coincides with the optically brightest arms (nos 4 and 5 in the notation of Baade¹¹) which contain most of the H II regions, as well as with the rings of high intensity seen at $\lambda 11$ cm in total power (Fig. 2 and ref. 10) and in the neutral hydrogen line⁹.

The radial distribution of the polarised intensity is shown in Fig. 3. In both the northern and in the southern half of M31 the bright ring extends from $R = 5$ to $R = 12$ kpc reaching a maximum intensity of $T_b^p \sim 8$ mK at $R = 8$ kpc. At some places in the ring ($\lambda_A = +20$ arc min, $\beta_A = -10$ arc min; $\lambda_A = -10$ arc min, $\beta_A = +10$ arc min) local maxima of $T_b^p \approx 18$ mK occur. Maxima due to polarised point sources are indicated in Fig. 1; the source with the highest polarised flux density (7 mJy corresponding to a degree of polarisation of 3%) is 5C3.107 at $\lambda_A = +7$ arc min, $\beta_A = +12$ arc min. In the ring several minima are seen coincident with large H II regions. At these positions the H II regions are probably in front of the synchrotron emission regions and depolarise the radiation.

In the north-east ($\lambda_A > 0$ arc min, $\beta_A < 0$ arc min), the north-west ($\lambda_A > 0$ arc min, $\beta_A > 0$ arc min), and the south-west ($\lambda_A < 0$ arc min, $\beta_A > 0$ arc min) the lines of maximum intensity (ridge lines) in polarisation as well as in total power (Fig. 2 and ref. 10) and H I (ref. 9) generally coincide with dust lanes. In the

south-east ($\lambda_A < 0$ arc min, $\beta_A < 0$ arc min), however, the ridge line in total power is less clearly coincident with dust lanes, and the ridge line in polarisation is shifted outwards with respect to the optical spiral arm.

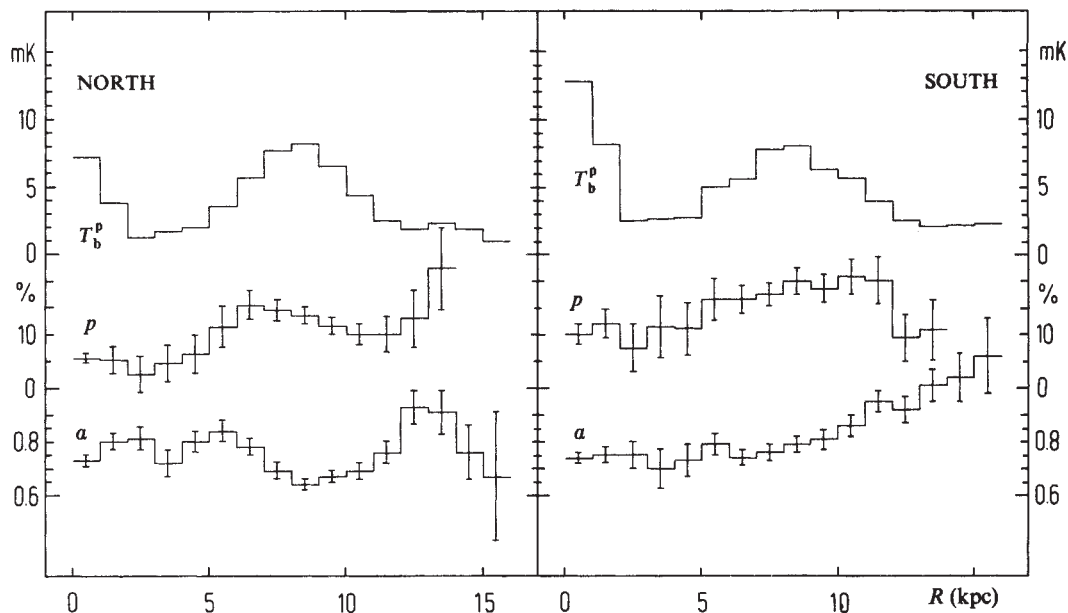
In the north-west, outside the bright ring, several weak maxima in T_b^p seem to line up along arm 5; all of these maxima coincide with dust regions. Conversely, the polarised feature at $\lambda_A = -60$ arc min, $\beta_A = +15$ to $+35$ arc min does not seem to be related to M31; this is probably a foreground irregularity within 500 pc of the Sun⁵.

Close to the centre of M31 an area with $T_b^p > 12$ mK is seen which seems to connect with the bright ring to the east but not to the west. This emission might originate in a region extending up to $z \approx 2$ kpc from the nucleus. If the western side were the closer to Earth, most of this emission would be seen in front of the plane to the east, while most would be depolarised by the plane to the west.

Figure 1 shows a well ordered pattern in the distribution of the polarisation angles. Near the southern major axis ($\lambda_A < -30$ arc min) the vectors are predominantly directed perpendicular to the spiral arm to within $\sim 30^\circ$. This orientation continues throughout the south-east quadrant, but near $\lambda_A = +5$ arc min, $\beta_A = -10$ arc min a clockwise rotation of the vectors of $\sim 90^\circ$ changes their preferential direction to along the spiral arm. In the south-west quadrant a similar clockwise rotation of the vectors occurs between $\lambda_A = -30$ and -10 arc min; the vectors remain orientated along the arm throughout the north-west quadrant and also follow the turn of the arm near the northern major axis.

In the absence of Faraday rotation the vectors are perpendicular to the projection of the magnetic field onto the plane of the sky. The orientation of the vectors perpendicular to the arm in the south-west and south-east then implies that the magnetic field is directed along the arms. Between $\lambda_A = -45$ and -30 arc min the angle ϕ between the arm and the line of sight varies between 16° and 60° , in which interval the rotation measure (RM) should increase by a factor of 2. As the perpendicular orientation of the vectors to the arm is maintained over

Fig. 3 Dependence of T_b^p , p and $\alpha_{408}^{2,700}$, as determined in circular rings in the plane of M31, on the distance R to the centre, shown for the north ($\lambda_A > 0$ arc min) and the south ($\lambda_A < 0$ arc min) separately.



this interval, an upper limit to the internal Faraday rotation on the major axis can be set of $\Delta\theta < 30^\circ$, or $RM < 42 \text{ rad m}^{-2}$. With a field component along the line of sight of $2 \mu\text{G}$ (ref. 12) and a path length through the arm on the major axis of $200 \text{ pc}/\cos 74^\circ$ the mean density of the thermal electrons is found to be $N_e < 0.04 \text{ cm}^{-3}$. This is comparable to the mean electron density of $\sim 0.03 \text{ cm}^{-3}$ derived by Ables and Manchester¹³ for the local region in our Galaxy.

The clockwise rotation of $\sim 90^\circ$ observed all over the northern part of M31 is believed to originate in the galactic foreground because: (1) the rotation is independent of ϕ ; and (2) it is unlikely that N_e in the entire northern part of M31 is at least three times higher than in the southern part because the north/south ratio of the thermal fraction is only ~ 1.5 (see Table 1). A rotation of -90° corresponds to $RM = -127 \text{ rad m}^{-2}$ which is in the range of rotation measures found by Simard-Normandin and Kronberg¹⁴ near M31.

Figure 1 shows that the average intensity of the polarised emission in the ring exceeds 10 mK near the minor axis but is only $\sim 3 \text{ mK}$ near the major axis. If the velocity field of the relativistic electrons is isotropic, such an effect is to be expected in the case of a magnetic field preferentially orientated along the bright ring: on the minor axis we are then looking perpendicular to the field ($\phi = 90^\circ$) and see high T_b^p , while on the major axis we are looking nearly along the field ($\phi = 16^\circ$) and find low T_b^p . Faraday effects would enhance this difference.

Thus both the variation of T_b^p in the ring of emission and the orientation of the vectors indicate that the magnetic field is preferentially aligned along the arms that form the ring.

In Fig. 1 the lengths of the vectors are proportional to the degree of polarisation, $p = T_b^p/T_b$, where T_b is the sum of thermal and nonthermal emission. The nucleus of M31 is $\sim 7\%$ polarised. The degree of polarisation is generally higher in the south than in the north. The maximum percentage observed is $p = 42\%$ at $\lambda_A, \beta_A = -10, +12$ and $-24, +12$ arc min in the south-west, and $\lambda_A, \beta_A = -12, -15$ and $-20, -15$ arc min in the south-east; in the north-east the highest percentage is $p = 37\%$ at $\lambda_A, \beta_A = +27, -5$ arc min. Several minima in the degree of polarisation are coincident with complexes of H II regions seen on the map of Pellet *et al.*¹⁵ (that is, $\lambda_A, \beta_A = +40, +6$ arc min; $\lambda_A, \beta_A = +12, -12$ arc min; $\lambda_A, \beta_A = -45, -9$ arc min) which may cause depolarisation.

To study the dependence of the degree of polarisation on distance to the centre, the average value of p was determined in circular rings in the plane of M31 so that the average of the angle ϕ is the same for every ring. The variation of p with R is shown in Fig. 3 for north and south separately. The variation of the

mean spectral index $\alpha_{408}^{2,700}$ (ref. 12) is also shown. In the north, p increases from 5% near the centre to 15% at $R \sim 7$ kpc on the inner side of the bright ring, and then decreases in the ring to 10% at $R \sim 11$ kpc. The spectral index also decreases markedly in the bright ring. This variation may be due to an increase of the thermal fraction in the ring, which will cause a decrease in p by a larger thermal contribution to T_b and an increase in Faraday depolarisation. In the south the situation is different: p and $\alpha_{408}^{2,700}$ are higher than in the north and show a constant increase with R across the bright ring.

Average values out to $R = 16$ kpc of p , $\alpha_{408}^{2,700}$, the thermal fraction f_{th} and the degree of polarisation corrected for the thermal contribution, p_n , are collected in Table 1 (ref. 12). The higher degree of polarisation in the southern half is only partly due to lower thermal fraction: there remains an excess of the polarised emission while there is no asymmetry in the total nonthermal emission.

Locally p_n will be much higher than the average values: at positions where $p = 42\%$, clearly $p_n > 42\%$. In the north-east similar high values of p_n are found if $f_{th} \sim 70\%$. In the case of a uniform field the intrinsic degree of polarisation of the nonthermal emission for $\alpha_n = 0.85$ is $p_i = 73.5\%$. Assuming that values of $p_n > 42\%$ are not affected by Faraday depolarisation, but that the difference between p_i and p_n is entirely due to irregularities of the magnetic field in the emission regions along the line of sight, the ratio of random field to uniform field is < 0.9 , corresponding to a degree of uniformity of $> 50\%$ on the scale of the beamwidth¹⁶. With the model of Segalovitz *et al.*⁴ a degree of uniformity $> 40\%$ and a ratio of random field/uniform field < 1.5 is derived. Thus the degree of uniformity of the magnetic field in M31 on a scale of 0.9×3.2 kpc is about three times higher than that found in M51 on a scale of 2.6×3.6 kpc (ref. 4). As the width and the separation of the arms as seen in the plane of the sky is comparable in these galaxies, the difference in the degree of uniformity of the field may be caused by a larger depolarisation across the approximately three times larger scale of the beam area used for M51.

Table 1 Average values for $R \leq 16$ kpc

Area	$\bar{p}$ (%)	$\bar{\alpha}_{408}^{2,700}$	$\bar{f}_{th}$ (%)	$\bar{p}_n$ (%)
Total	14.6 ± 1.5	$0.76 \pm .05$	30 ± 5	21 ± 3
North	11.7 ± 1.4	$0.71 \pm .05$	35 ± 6	18 ± 3
South	18.3 ± 1.9	$0.82 \pm .05$	24 ± 4	24 ± 3

An estimate of the Faraday depolarisation and a study of the intrinsic polarisation angles in the bright ring of M31 can be made when our observations at λ 18 cm (underway) and λ 6 cm (planned) are complete.

Received 26 August; accepted 22 November 1979.

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Peculiarities of binary galaxies

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A recent analysis of a sample of close binary galaxies¹ by White and Valdes² has revealed some peculiar features: in particular, they tend to be brighter than field galaxies. Binary galaxies are important because the relative simplicity of the two-body problem in dynamics should make it possible to obtain good estimates of the masses of paired galaxies. This would help in determining whether there are massive haloes of dark, unseen matter, and in the problem area of galaxy formation. Much effort has therefore been expended (ref. 3 and refs therein), but conclusions about the existence of massive haloes and about the mass-to-light ratios have varied. Because we do not have complete information, various statistical assumptions must be made in these analyses. Detailed arguments² suggest that one of these assumptions has been in error in previous work. We present a test which supports this claim. (The sample used here and in ref. 2 seems to be the only set of paired galaxies with quantitative selection criteria and velocity information for an unbiased subsample.) Furthermore, it was shown² that binary galaxies are drawn from a luminosity function which has a characteristic luminosity brighter than that for field galaxies by almost one whole magnitude. We discuss one of the possible explanations and some suggestive evidence.

The basic observational information available includes the apparent magnitude of the two galaxies, their redshifts, and their angular separation on the sky, θ . The (probably reasonable) assumption that the centre of mass of the system partakes of the Hubble flow enables these data to be converted to absolute luminosities, a line-of-sight velocity difference, and a projected rectilinear separation r_p (see ref. 3). Further analysis of a single pair would require knowledge of the angle between the true separation vector and the line-of-sight, ϕ , the orbital eccentricity, and the orbital phase. Since we do not have these data, we must make assumptions about them. Such assumptions are usually statistical in nature, because samples usually comprise many pairs. Thus, for example, one can assume a distribution of eccentricities in the sample. This seems to indicate the need for low eccentricity, but is apparently not crucial to mass determinations³.

Another important assumption is the distribution of the angle ϕ , which relates the true separation r to the projected separation r_p by $r_p = r \sin \phi$. Previous analyses^{3–5} have used a distribution $N(\phi) d\phi \propto (\sin \phi)^\alpha d\phi$ with an exponent $\alpha = 0.5$, derived from the data. The recent analysis of White and Valdes², however, uses the covariance function to derive $\alpha = -(2 - \gamma)$ for a two-point function $\xi(r) = (r_0/r)^\gamma$. The accepted value for γ of 1.8 thus leads to $\alpha = -0.2$. It seems that the earlier authors erroneously assumed that pairs isolated from correlated objects have the orientation distribution appropriate to pairs isolated from an uncorrelated population. The change from $\alpha = 0.5$ to $\alpha = -0.2$ increases mass determinations, made using Turner's method³, by about 40%, and can affect rank-sum techniques⁴ by as much as a factor two² (although the use of overlapping bins in this analysis may have introduced other sources of uncertainty). It is therefore very important to decide which exponent is correct.

It was suggested by D. Lynden-Bell (personal communication) that it should be possible to use a test analogous to the $V/V_{\max}$ test used to study the space distribution of quasars⁶. For the present sample, we consider the angular separation θ and the maximum separation that the pair could have had and still be included in the sample, $\theta_{\max}$. This latter angle is either one-fifth of the distance to the third galaxy nearest the pair, or 8 arc min, whichever is the smaller (see the selection criteria of ref. 1). Figure 1 shows a plot $\theta/\theta_{\max}$ against $\theta_{\max}$ for the full sample of 156 pairs¹. (Because the values for θ are the precise measurements of Turner, whilst the values for $\theta_{\max}$ come from neighbour calculations using the positions given in the Zwicky catalogue, some ratios are greater than one. This increases the scatter without introducing any significant problems).

Assuming a power-law distribution as above gives $n(\theta)d\theta \propto \theta^{-\alpha}d\theta$, which then predicts that the average value $\langle \theta/\theta_{\max} \rangle$ is $[(1 + \alpha) \pm N^{-1/2}(1 + \alpha)^{1/2}(3 + \alpha)^{-1/2}]/(2 + \alpha)$ for a sample size of N . For 156 pairs, the data give $\langle \theta/\theta_{\max} \rangle = 0.52 \pm 0.02$; the predictions are 0.33 ± 0.02 ($\alpha = 0.5$) and 0.55 ± 0.02 ($\alpha = -0.2$). This suggests that $\alpha = -0.1 \pm 0.1$. Various subsamples have been used in different works based on binary galaxies^{2,3,7,8}, and there is no obvious systematic variation in the values of $\langle \theta/\theta_{\max} \rangle$ for them. In addition, there does not seem to be any clear dependence on $\theta_{\max}$ for any of the sets. The recent argument is thus clearly supported.

The second puzzling aspect is the apparently increased luminosity of paired galaxies. An obvious explanation is that the interaction of the two galaxies induces star formation and thus

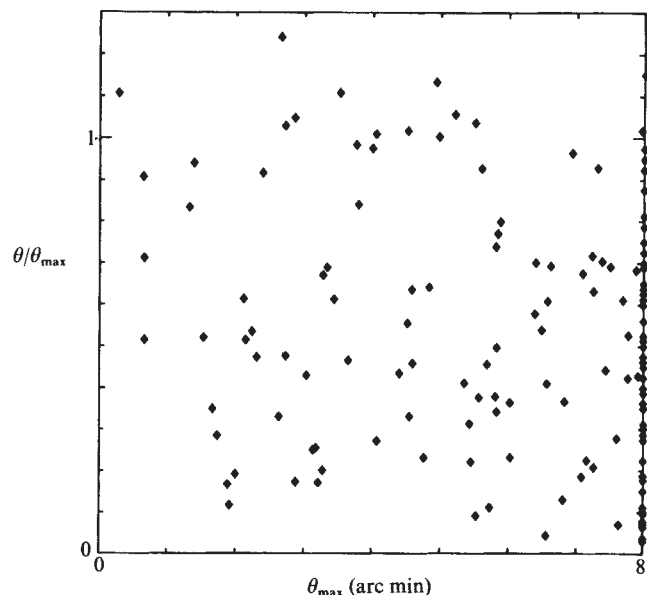


Fig. 1 The ratio $\theta/\theta_{\max}$ plotted against $\theta_{\max}$ for the 156 pairs of ref. 1.

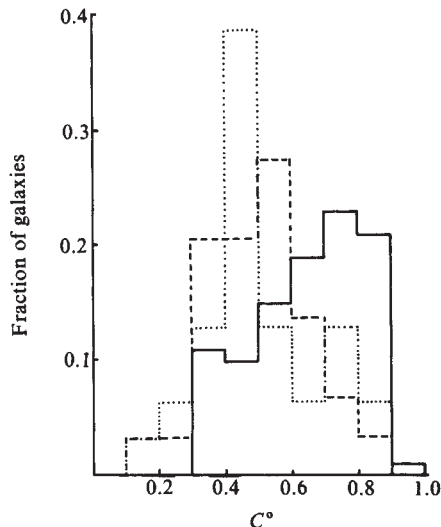


Fig. 2 The distribution of the absorption corrected, integrated $B-V$ colour index C° . solid line: 100 randomly chosen non-elliptical galaxies. Dashed line: close spiral-spiral pairs. Dotted line: wide spiral-spiral pairs.

makes them brighter. This idea is supported by work on normal and peculiar galaxies⁹. As the method by which star formation might be enhanced is not clear, possible correlations between separation, morphological type, and/or luminosity excess, are not well predicted.

An excellent check would be to consider the colours of the binary galaxies, since one would expect that sufficient star formation to increase the luminosity to the required extent would make the galaxies quite distinctly bluer⁹. These data are not available for the Turner sample, but some suggestive evidence comes from a survey (B. J., unpublished) based on the data of Holmberg¹⁰, split into 'wide' pairs, 'close' pairs, and the field. By 'close' is meant a pair whose separation is of the order of or less than the sum of the face-on diameters of the components. 'Wide' comprises systems which are essentially not touching.

Figure 2 compares three histograms showing the distribution of the absorption-corrected integrated $B-V$ colour index C° . The solid line is for 100 randomly chosen non-elliptical galaxies; the dashed line is for close spiral-spiral pairs; the dotted line is for wide spiral-spiral pairs. The mean colour index for the three classes goes from 0.64 (field) to 0.50 (wide) and to 0.49 (close), with r.m.s. deviations of 0.16, 0.17 and 0.16 respectively. In

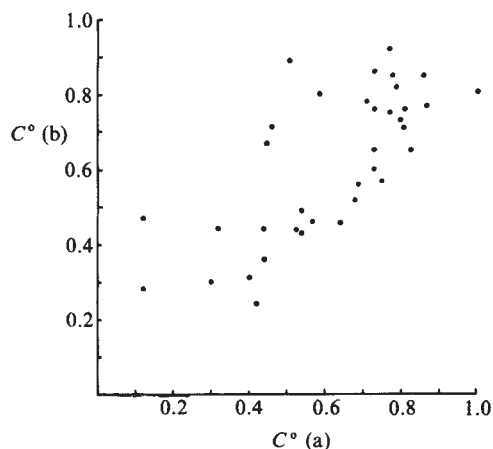


Fig. 3 The correlation between the colour index of the brighter (or larger) galaxy, $C^\circ(a)$, and that of its companion, $C^\circ(b)$.

addition, it is clear that the distributions for the paired galaxies are skewed to the blue of the 'ordinary' galaxies.

That this colour change is due to interaction is strongly supported by the correlation between the colours of the two components. In Fig. 3 the colour index for the brighter (or larger) galaxy, $C^\circ(a)$, is plotted against that of its companion, $C^\circ(b)$, for the spiral-spiral pairs. The correlation coefficient is 0.7: red (blue) galaxies tend to have red (blue) companions. (There is also a suggestive correlation of morphological type⁵.)

Let L_B and L_V be the B and V luminosities of a galaxy before interaction, and let l_B and l_V be the contributions from stars formed as a consequence of the interaction. Then $L_V/L_B = 10^{0.4C}$ and $l_V/l_B = 10^{0.4C_*}$, where C is the colour index of the pre-interaction galaxy and C_* is the mean colour index of the newly created stars. The colour change is then

$$\Delta C = -2.5 \log_{10} \left\{ \frac{1 + 10^{0.4(C-C_*)} l_V/L_V}{1 + l_V/L_V} \right\} \quad (1)$$

and the associated magnitude changes are $\Delta M_V = -2.5 \log_{10}(1 + l_V/L_V)$ and $\Delta M_B = \Delta M_V + \Delta C$. Figure 4 shows a few curves of $-\Delta C$ plotted against l_V/L_V , labelled by their values of $C - C_*$. The upper scale gives $(-\Delta M_V)$, and some curves of constant ΔM_B are also shown.

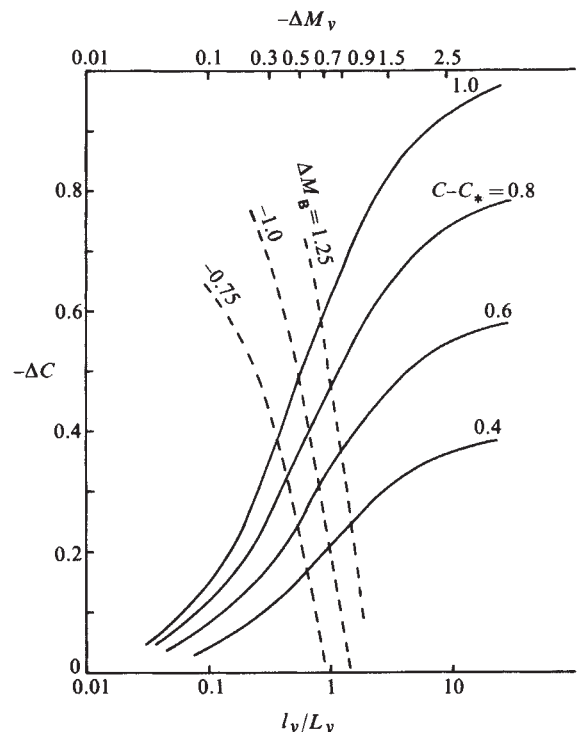


Fig. 4 The relation between the colour change ΔC and the magnitude changes ΔM_V and ΔM_B , for different values of $C - C_*$ (see equation (1)).

Figures 2 and 3 here, and Fig. 1 of ref. 9, suggest that a colour change of about 0.3–0.5 mag is required. The value of C_* to take is given roughly by the time elapsed since the interaction: choose the stars that spend that amount of time on the main sequence. (The finer details depend on the mass function of the stars.) If the galaxies interacted between 10^8 and 10^9 yr ago, C_* is in the range -0.15 to $+0.15$. An initial galaxy of C about 0.7 therefore has $C - C_*$ in the range 0.55 to 0.85. Figure 4 then indicates a value for ΔM_V of 0.5 to 0.8 mag, and for ΔM_B of 0.75 to 1.25 mag.

Although the samples are selected differently^{1,10}, the same physical processes should occur in both. In addition, it is not certain which stage in a binary galaxy's life is most propitious for induced star formation and the ages of the binary systems are

difficult to determine. Although the orbital times for the Turner sample are so short that significant interaction (of a dynamical sort at least) seems inevitable^{7,11}, it also seems that the disk galaxy pairs were probably not dynamically isolated from other galaxies at the time of their formation⁸. This makes consideration of the induced star formation yet more complex.

However, the evidence presented here shows that significant colour changes are induced by interaction, and that these are of such a magnitude and take place over such a time scale that the B magnitude of a galaxy would increase by about one magnitude, as required by White and Valdes². To confirm this, better data are required—particularly a detailed study of the colours of the Turner pairs.

Note added in proof: The extension of the covariance function to small scales¹² adds more weight to the arguments here and in ref. 2.

Received 28 August; accepted 15 October 1979.

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Spectral evidence for a carbonaceous chondrite surface composition on Deimos

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The good match between the reflectance spectra of Phobos and Ceres, an asteroid inferred to have a composition analogous to carbonaceous chondrites because of its low density, $\sim 2.2 \text{ g cm}^{-3}$ (ref. 1), and spectral signatures that include a water of hydration band centred at $3.0 \mu\text{m}$ (refs 2, 3), has led to the conclusion⁴ that the surface composition of the inner martian satellite is similar to that of carbonaceous chondrites. Pollack *et al.*⁵ independently reached the same conclusion by comparing Phobos' spectral albedo with reflectance spectra of meteorites and basalts. Mass and density estimates ($\sim 2 \text{ g cm}^{-3}$) for the satellite were made based on the perturbations on the orbit of Viking 1 by Phobos, during a series of close encounters⁶. The density of Phobos is consistent with the same composition as that inferred for its surface. Among materials expected to be common in the Solar System only ices or water-rich carbonaceous chondrites (20% H_2O by weight in type C1) have densities as low as Ceres and Phobos⁷. The compositional results have forced a re-evaluation of hypotheses concerning Phobos' origin. Scientific opinion now seems to favour an origin by capture of a body that came too close to Mars⁸. The analysis of data on Deimos reported here reveals a spectral reflectance very similar to that of Phobos, indicating a surface composition analogous to that of carbonaceous chondrites.

High-resolution pictures of Deimos obtained by Viking Orbiter 2 in recent close flybys showed a surface that appears to be morphologically different from that of Phobos⁹. Although the two satellites have equal densities of impact craters, on Deimos craters 50 m in diameter are filled in with fine-grained material,

accounting for the relatively smooth appearance of the outer martian moon. Grooves are found over most of Phobos, but are conspicuously absent from the surface of Deimos. Why is the surface of Deimos but not Phobos mantled with large amounts of loose material, including clusters of house-sized blocks? One possibility is that the surfaces of the two satellites have significantly different mechanical properties. For example, an impact might produce a larger fraction of low-velocity fragments on Deimos than it would on Phobos. A second explanation is that it might be easier to knock material off Phobos than off Deimos, because Phobos is closer to Mars¹⁰. We will evaluate the first hypothesis by comparing the surface composition of the two satellites, that is by comparing the UV-visible reflectance spectrum of Deimos with that of Phobos.

The UV-visible reflectance spectrum of Deimos is compiled from the following sources: Mariner 9 UV spectrometry (UVS) and Canopus star tracker photometry, and ground-based colorimetry and polarimetry. Mariner 9 UV spectra of Deimos at a resolution of 1.5 nm were obtained on the 73rd revolution of the spacecraft around Mars. The phase angle of observation was 22° . Analysis of the Deimos spectra follows the procedure developed for Phobos⁴ with the exception that a broader bandwidth was used, because the signal-to-noise ratio for Deimos was not as good as that for Phobos. Both preflight laboratory and inflight stellar calibrations show the absolute uncertainty of the UVS measurements to be $\pm 20\%$, with a relative uncertainty of about $\pm 7\%$. The relative uncertainty is shown in Fig. 1 as error bars.

Zellner and Capen¹¹ determined the slope of Deimos' polarisation curve at phase angles between 15° and 40° . Interpreting this slope on the basis of the new slope-albedo law^{12,13}, we arrive at a geometric albedo for Deimos in blue light of ≤ 0.057 (arrow in Fig. 1). They also made photoelectric observations of Deimos and derived a visual (V) geometric albedo of 0.070 ± 0.008 , blue-visual colour index $B - V = 0.65 \pm 0.03$ and ultraviolet-blue colour $U - B = 0.18 \pm 0.03$, and phase coefficient $\beta_v = 0.036 \pm 0.05$ magnitude per degree for phase angles $< 20^\circ$.

Ground-based absolute photometry of the martian moons is very difficult due to their proximity to Mars (hence interference by light scattered from a bright primary). Photometric observations of Phobos from the martian surface with Viking Lander cameras did not have such a handicap⁵. We, therefore, made a new determination of Deimos' geometric albedo, using observations of the satellite made by the Mariner 9 Canopus star tracker (CST). Viewing Deimos from a Mars-orbiting platform avoids the problems caused by halation from Mars. Details of CST functions and data analysis are given elsewhere¹⁴⁻¹⁷. We shall first test CST data analysis on Phobos.

Following the procedure of Bowell and Zellner¹⁸, we obtained Phobos' geometric albedo by extrapolating 83 CST measurements to a phase angle of 0° , using our own phase coefficient for angles $72-98^\circ$ (ref. 19) and Noland and Veverka's β_v for $0-72^\circ$ (ref. 20). [Similar values for the phase coefficients have been obtained by Klaasen *et al.*²¹.] We assumed that Phobos' phase coefficient in the unobserved range of phase angles $0-18^\circ$ is the same as that for the range $18-72^\circ$, because its phase curve is closely similar to that of Earth's moon (ref. 19), which has a nearly constant slope in the $0-70^\circ$ region²². The average of 83 geometric albedo determinations is 0.046, with an absolute uncertainty of about ± 0.01 . It is slightly smaller than a similar broadband geometric albedo found from Viking lander imaging, 0.05 ± 0.01 (ref. 5). The small difference is due to different assumptions made in analysing the Viking lander data. When extrapolating Viking lander observations, made at 35° phase, to 0° phase, we had assumed that Phobos' phase coefficient between 0° and 22° is the same as that of Deimos^{4,5}. Because of the grossly different surface roughness of the two objects we now prefer a lunar-like phase curve between 0° and 72° . Using the same β_v as in the CST analysis we find that Viking Lander camera albedos for Phobos to be near 0.045 (see Fig. 1). The CST albedo for Phobos is plotted as a continuous line. The

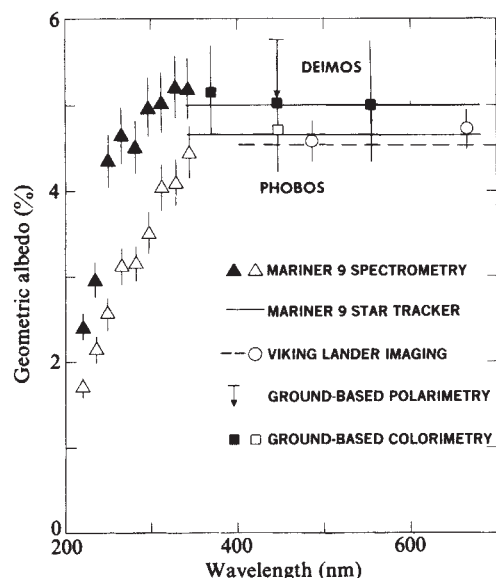


Fig. 1 The spectral albedo of the martian satellites. UV geometric albedos at 15-nm band centres: $\blacktriangle$, Deimos; $\triangle$, Phobos. Photometry: $\circ$, narrow band; ---, broad band. Polarimetry $\downarrow$. Colorimetry: $\blacksquare$, Deimos; $\square$, Phobos.

lengths of the lines indicate the bandwidths of the CST and Viking Lander camera broadband photometry. The CST has a blueish response so that CST magnitudes are similar to the commonly used photographic magnitudes. Kuiper's $B-V$ colour index for Phobos has been converted into a blue albedo after normalising to the Viking Lander imaging results⁵ (open square in Fig. 1).

Following the same procedure as that for Phobos, analysis of 12 CST measurements yielded an average geometric albedo of 0.050, with an absolute uncertainty of about ± 0.01 for Deimos. Zellner and Capen's colour indices were converted to UBV geometric albedos after normalising to the CST value. B and V albedos are the same for both Phobos and Deimos as the satellites have the same $B-V$ colour as the Sun. Viking Lander photometry has an absolute accuracy of $\pm 20\%$ and relative accuracy of $\pm 5\%$ (error bars). Ground-based data are accurate to ± 0.01 (error bars). The ratio of Deimos' geometric albedo to Phobos' is now 1.1, as required by relative photometry of the satellites²⁰.

To a first approximation the reflectance spectra of Deimos and Phobos are very similar: both have low and flat reflectivities in the visible, dropping off sharply in the UV. Searches through spectral signatures of asteroids of known compositions and laboratory reflectance measurements of rocks, minerals and meteorites yielded carbonaceous chondrites as best matches for the spectral albedo of Phobos³⁻⁵. Only carbonaceous chondrites possess all three required spectral characteristics: low albedo, flat visible-IR reflectivity, with sharp downward bend in the UV. On the basis of a good match between the reflectance spectra of Deimos and Phobos, and with the composition of Phobos known to be similar to that of carbonaceous chondrites, it is reasonable to believe that the surface composition of Deimos is also similar to that of carbonaceous chondrites. Our identification of a carbonaceous chondrite composition for Deimos is consistent with the implications of a recent mass estimate for the satellite based on the perturbations on the orbit of Viking Orbiter 2 by Deimos, during a series of close encounters. This mass estimate yields preliminary densities that are equal to or less than the density of Phobos²³.

Why then do the surfaces of the martian satellites appear to be morphologically different? Any explanation requiring Phobos and Deimos to have surfaces with different mechanical strength seems to be unrealistic in view of their similar compositions and densities; instead, the states of the satellites' surfaces are probably due to their different orbital history.

We thank Ian Gatley, and members of the Mariner 9 UVS and Guidance and Control teams for their support. Funds for this research are derived in part from a NASA contract (Mars Data Analysis Program).

Received 29 May; accepted 16 November 1979.

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High time resolution riometer and X-ray measurements of conjugate electron precipitation from the magnetosphere

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In this letter we describe the comparison of cosmic noise absorption data recorded by a riometer at Siple Station, Antarctica (76°S ; 84°W ; $L \sim 4.2$) with simultaneous balloon measurements of bremsstrahlung X rays from the conjugate region near Roberval, Canada (48.3°N ; 72.1°W). Both the cosmic noise absorption and the production of X rays are caused by the precipitation of energetic electrons ($E \geq 15$ keV) from the magnetosphere. The time resolution and spatial coverage of the measurements enable us for the first time to show the similarity and simultaneity of rapid fluctuations of electron precipitation at ionospheric conjugate points. Furthermore, the data suggest that fast-response riometers are capable of resolving temporal structures in electron precipitation on a time scale which approaches that of electron microbursts (of the order of 1 s). The relative simplicity of riometers for the measurement of precipitating particle effects opens up important new avenues of research in studies of upper atmosphere ionisation phenomena.

The Siple riometer operates at 30 MHz and has a response time of 0.25 s to increases or decreases in signal intensity¹. It utilises a zenith-directed dipole antenna with half-power at 30° . The antenna pattern projected to 100 km altitude covers a circular area of approximately 120 km diameter. The riometer data and the output of a three-axis fluxgate magnetometer are digitised at 2-s intervals and written in computer-compatible format on magnetic tape^{2,3}. The X-ray measurements in the conjugate region were made at 35 km altitude with a zenith-directed uncollimated sodium iodide scintillation counter. An energy spectrum covering the range from 25 to 500 keV in seven

differential channels is obtained with a time resolution of 0.01 s. The effective response of this detector projected to 100 km altitude covers a circular area equal to that of the Siple riometer. Calculations place the Siple conjugate point within 60 km of Roberval^{4,5}. At the time of measurement, the balloon was located 30 km northeast of Roberval. Thus, portions of the effective areas of balloon and riometer gave overlapping coverage of the same field line(s). Uncertainty in the relative timing of the measurements in opposite hemispheres was ≤ 1 s.

A 1-min segment of X-ray, riometer and magnetometer data obtained during a microburst precipitation event on 9 July 1975 is presented in Fig. 1. Panel *a* illustrates the X-ray counting rate of the 45–65 keV energy channel at a resolution of 0.1 s. At this time, individual microbursts⁶, the spikes of 0.2-s duration spaced 0.5–1 s apart, were occurring in groups separated by 5–10 s. The same X-ray data are plotted in Fig. 1*b* as 2-s averages, a reduction by a factor of 20 in time resolution. The Siple riometer and *D*-component magnetometer data, both limited in this event to the 2-s sampling rate, are shown in Fig. 1*c* and *d*, respectively.

It is evident on comparing *b* and *c* of Fig. 1 that each increase in X-ray counting rate at Roberval was followed several seconds later by an increase in cosmic noise absorption at Siple. Absorption variations as small as 0.02 dB are significant (the total ionospheric absorption caused by electron precipitation above Siple at this time was about 2 dB). At this resolution (2 s), the intensity-time profiles of absorption and X-ray pulses show some striking similarities. In general, however, the absorption pulses are somewhat broader than the associated X-ray pulses.

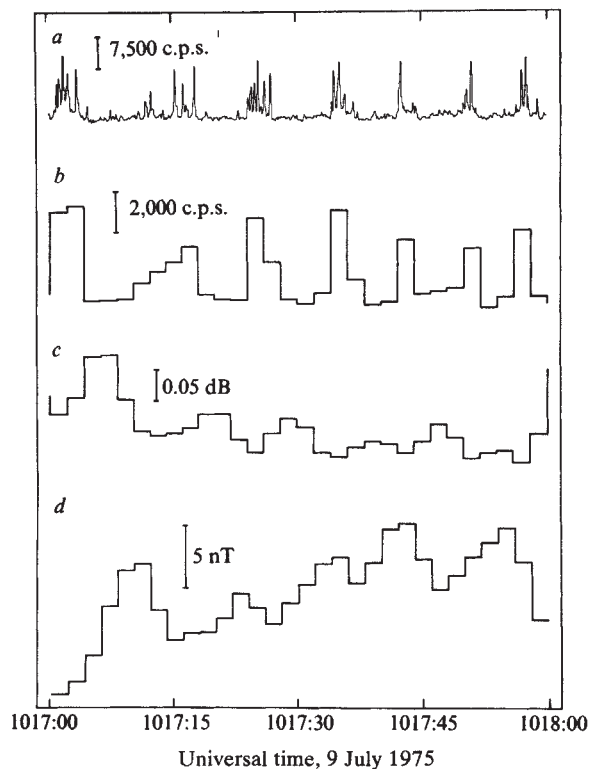


Fig. 1 *a*, Atmospheric bremsstrahlung X rays produced near Roberval, Canada ($L \approx 4.1$) by the precipitation of energetic electrons during a magnetic substorm. Individual microbursts are well resolved at 0.1-s resolution. *b*, The same X-ray data as in (*a*) but with a factor of 20 less resolution. The microburst structure is no longer apparent. *c*, Ionospheric absorption fluctuations at Siple Station, Antarctica (magnetically conjugate to Roberval) obtained by a 30-MHz riometer with resolution (2 s) equal to that of the X-ray data in *b*. Increasing absorption is upward. A time-delayed correlation (absorption lags X rays by ~ 4 s) is evident. *d*, Variations of the *D* component of the surface magnetic field at Siple at 2-s resolution. LT = UT - 5 hours.

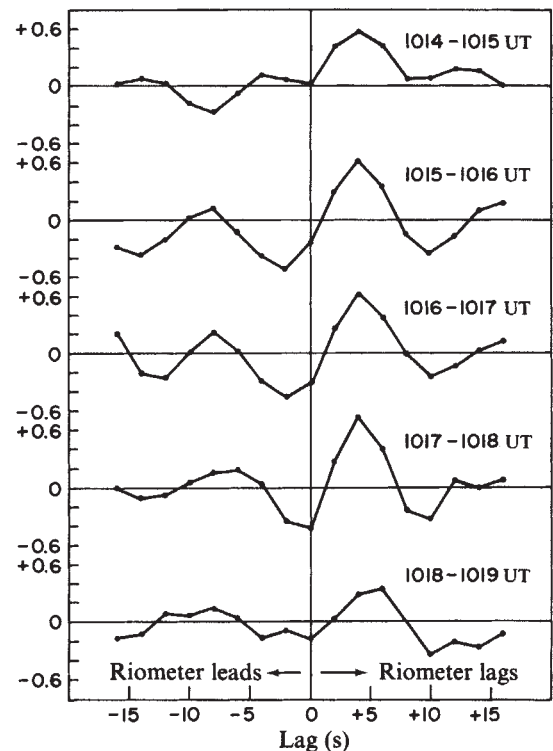


Fig. 2 Cross-correlation of 2-s resolution Siple riometer and Roberval X-ray data for consecutive 1-min segments. The correlation peak at +4 s exceeds the 99% confidence level in each of the first four segments.

This is to be expected from considerations of ionospheric response time even if the precipitation were identical at the conjugate points.

Magnetic variations on a comparable time scale also occurred. Further details of the accompanying magnetospheric substorm and of the association of ultra low frequency (ULF) magnetic variations and absorption fluctuations have been given previously^{2,7}.

The data in Fig. 1 represent part of an extended period of disturbance. Figure 2 shows the results of cross-correlating the Roberval X-ray and Siple riometer data (2-s resolution) for consecutive 1-min segments between 1014 and 1019 UT. The peak at +4 s (riometer lags X rays) is confirmation that rapid fluctuations of electron precipitation are highly correlated at magnetic conjugate points. Approximately 1.8 s of the time delay is due to a phase shift introduced by a low-pass anti-aliasing filter in the riometer data system. For the data sets 1015–1016 and 1016–1017 UT, the probability of obtaining the observed correlation by chance is less than 0.001. For 1017–1018 UT the probability of obtaining the observed correlation by chance is less than 0.0001. For the interval 1015–1018 taken as a whole the probability is less than 1 in 10^8 that the correlation could have occurred by chance.

Previous studies of riometer pulsations accompanying X-ray⁸ and luminosity⁹ pulsations, using data from one end of a field line only, have found time delays of peak absorption of several seconds. This suggests, therefore, that the ≈ 2 s by which the absorption pulses lag the X-ray pulses in the present event may be accounted for by ionospheric response. Our investigation of this point will be presented elsewhere¹⁰.

Because the data sampling rate did not take full advantage of the response capability of the Siple riometer, it was not possible to verify the presence of microburst structure in absorption. However, it should be possible to achieve finer time resolution. Considerations of ionospheric response suggest that absorption fluctuations caused by microbursts could be observed if the ionospheric time constant⁸ [$\tau \approx (2\alpha N_e)^{-1}$, where N_e is the electron density and α is the effective recombination coefficient]

were ≤ 5 s, given the sensitivity (~ 0.02 dB) and instrumental time constant (~ 0.1 s) that can be achieved with riometers. Ionospheric time constants of this order may apply in the D region during absorption events associated with some magnetic storms and substorms.

The research at the University of Maryland was supported by NSF grants DPP74-01704, DPP76-82041 and ATM77-22937. Additional support for balloon operations in Canada was provided by the Office of Naval Research (contract N00014-67-A-0239-0033). Bell Laboratories acknowledge the NSF for logistics support for the Siple measurements. We thank Dr H. Chivers for cooperation in the riometer data acquisition.

Received 4 September; accepted 13 November 1979.

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Charge dependence of pion production in heavy ion collisions

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Benenson *et al.*¹ measured the pion production cross-section in nuclear heavy ion collisions in the energy range 100–400 MeV per nucleon with the aim of determining whether collective effects associated with the larger number of nucleons were present in the pion field. An instability in the pion field, such as the putative pion condensation phase transition², would give rise to an enhanced number of physical pions. The expected production cross-section, in the absence of collective effects, is found from two simple models. One of these is a statistical model, in which the number of pions is calculated from a statistical equilibrium. At the other extreme, the nucleons in the target and projectile are assumed to be free and independent, except for their Fermi momentum, and create pions by two-body collisions. The experimental cross-section at the lowest energy was found to be larger than either model by at least an order of magnitude³, suggesting that collective effects are indeed present. Interestingly, the cross-section had unexpectedly strong dependence on the charge of the pion produced. At the highest energy studied, 400 MeV per nucleon, many more π^- than π^+ were produced in the forward direction. At the lowest energy studied, 100 MeV per nucleon, the relative yields are reversed, with more π^+ produced. The experimental ratios of π^- to π^+ ,

$$R_{-/+} = \frac{d\sigma_{\pi^-}/d^3p}{d\sigma_{\pi^+}/d^3p}$$

are quoted in Table 1. Benenson *et al.*¹ explain the π^- enhancement in terms of the Coulomb distortions of the pion wavefunctions. Here I include in the same framework an explanation of the low energy result.

My starting point for the pion production rate is the formula

$$W = 2\pi \sum_i \langle i | H_{\pi N} | f \rangle^2 \rho_i$$

Table 1 Experimental ratios of π^- to π^+

E_{proj} (MeV per nucleon)	Pion kinetic energy (MeV)	$y_{\pi^-} - y_{\text{proj}}^*$	$R_{-/+}$
~ 100	34	0.23	0.7
	54	0.40	0.6
	76	0.54	0.5
400	34	-0.20	3.0
	54	-0.04	10.0
	76	0.11	2.5
	123	0.36	1.4
	155	0.49	1.2

* Rapidity, the Lorentz-additive measure of velocity, is defined as $y = \frac{1}{2} \ln(E + pc)/(E - pc)$.

where ρ_i is the density of final states and $H_{\pi N}$ is the pion creation part of the nuclear hamiltonian. The ρ_i is a product of density of pionic states ρ_i^π , and density of nucleonic states ρ_i^N . Normalising the pion wavefunction to plane waves at infinity, the pionic density of states is the usual $\rho_i^\pi = \frac{d^3p}{(2\pi)^3}$. The transition matrix element $\langle i | H_{\pi N} | f \rangle^2$ will be proportional to the probability of these normalised wavefunctions in the collision region, $|\phi_{\pi^*}|^2$. Benenson *et al.*¹ compute the charge dependence as

$$R_{-/+} \approx \frac{|\phi_{\pi^-}(R)|^2}{|\phi_{\pi^+}(R)|^2}$$

where ϕ_{π^*} contains the Coulomb distortion of the projectile. Thereby the large effect seen for pion velocities close to the beam velocity is qualitatively explained. The effect of the nuclear density of states will now be included. To describe ρ_i^N , it is assumed that there is a thermal equilibrium among nucleons. The final state density then depends on pion energy and charge according to

$$\rho_i^N \sim \exp[-(E_{\pi^*} \mp (\mu_p - \mu_n))/kT] \quad (1)$$

Here kT is the temperature of the nucleons, and μ_p and μ_n are the chemical potentials of the protons and neutrons. In view of the relatively small number of nucleons involved in the collision, and the fact that the pions are most likely to be created at the initial stages of the collision, before any sort of equilibrium is established, the statistical assumption may be questioned. However, it is a fact that energy spectra tend to be exponential, with a coefficient close to the 'temperature' calculated as below⁴. Thus we at least find phenomenological justification for equation (1).

$R_{-/+}$ is now compared at the two energies, for the same velocity of the pion with respect to the projectile. The Coulomb distortion effects should be nearly the same, so the ratio of $R_{-/+}$ would be given by

$$R_{-/+}/R'_{-/+} \approx \exp \left[2(\mu_p - \mu_n) \left(\frac{1}{kT'} - \frac{1}{kT} \right) \right]$$

In the system studied experimentally, $^{20}\text{Ne} + \text{NaF}$, the chemical potentials differ mainly because of the Coulomb field. Assuming that the pion production takes place in an overlap region between the colliding nuclei, the difference between chemical potentials may be estimated as

$$\mu_p - \mu_n = \frac{Ze^2}{R} \approx \frac{20(1.44)}{3.3} \text{ MeV} \approx 8.7 \text{ MeV}$$

To estimate the temperature at 100 MeV per nucleon projectile energy, note that the energy available in the centre of mass, per nucleon, is ~ 25 MeV. With Boltzmann statistics, this implies a temperature of $\frac{2}{3}(25) = 16.7$ MeV. A more detailed calculation taking into account such things as quantum statistics and binding energy effects yields a temperature of 13.5 MeV. Similar considerations for 400 MeV per nucleon yield a temperature of

55 MeV. Then for a given pion velocity with respect to the projectile, we expect a diminution of π^- at the lower energy of

$$R/R' \approx \exp\left(17.4\left(\frac{1}{55} - \frac{1}{13.5}\right)\right) \approx 0.37$$

This agrees with the experimental ratio $0.5/1.2 \approx 0.42$ found for pions of high rapidity.

From these considerations, it seems that $R_{-/+}$ will be a useful probe of the nucleonic density of states in the early stages of heavy ion collisions at lower bombarding energies, and seems to be conveniently interpretable as a temperature. This complements the usefulness of $R_{-/+}$ at higher energies as a probe of the geometry of the collisions, via the dependence on the Coulomb distortions.

I thank W. Benenson for discussions, and the NSF for support under grant no. PHY-7620097.

Received 18 September; accepted 22 November 1979.

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Improved planar solar convertor based on uranyl neodymium and holmium glasses

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The use of uranyl doped glasses for solar energy conversion and concentration was discussed by Reisfeld and Neuman¹. While the UO_2^{2+} ion is an efficient energy convertor, its maximum emission peaking around 500 nm does not coincide with the maximum sensitivity of the existing solar cells which is around 700–1,000 nm. The use of Nd^{3+} doped glasses for solar energy collectors was described by Levitt and Weber². While the emission of Nd^{3+} peaking at 880 nm ($^4\text{F}_{3/2} \rightarrow ^4\text{I}_{9/2}$) and at 1.06 μm ($^4\text{F}_{3/2} \rightarrow ^4\text{I}_{11/2}$) is ideal for matching with the maximum sensitivity of solar cells, its absorbance arising from the forbidden transition $4f-4f$ is low and the overlap of the absorption spectrum of Nd^{3+} with the solar spectrum is rather weak. To increase the operational efficiency of solar collectors, a maximum overlap between the absorption spectrum of the collector and the solar spectrum is required³. In this report we suggest that glasses doped by uranyl and neodymium and uranyl and holmium increase the response to the solar spectrum, thus increasing the collection efficiency, and convert the absorbed light in the wide spectral range into the narrow fluorescence band at 1.06 μm and 880 nm in uranyl-neodymium glasses and 660 nm ($^5\text{F}_5 \rightarrow ^5\text{I}_8$) and 750 nm ($^5\text{F}_4, ^5\text{S}_2 \rightarrow ^5\text{I}_7$) in the uranyl-holmium co-doped glasses.

Energy transfer from $\text{UO}_2^{2+} \rightarrow \text{Nd}^{3+}$ in barium crown glasses was discussed by Gandy *et al.*⁴ and Melamed *et al.*⁵ for small concentrations of UO_2^{2+} in the glasses. The use of energy transfer for lowering the laser threshold in rare earth doped glasses is a well-known phenomenon⁶. Here we present quantitative calculations on energy transfer between $\text{UO}_2^{2+} \rightarrow \text{Nd}^{3+}$ and $\text{UO}_2^{2+} \rightarrow \text{Ho}^{3+}$ at a much higher concentration range than in ref. 4. This higher concentration range is suitable for solar energy conversion. The absorption, excitation and emission spectrum of UO_2^{2+} doped phosphate glasses may be found in ref. 1.

The excitation spectrum of the $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{11/2}$ (1.06 μm) transition of a glass doped by 2 wt % Nd^{3+} and co-doped by 2 wt % Nd^{3+} + 1 wt % UO_2^{2+} in the spectral range 360–500 nm

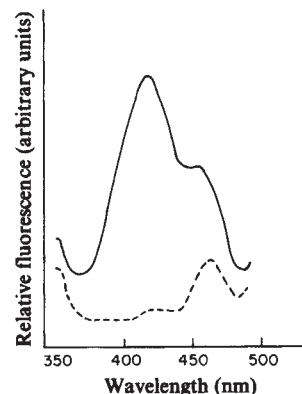


Fig. 1 Excitation of Nd^{3+} (---) and $\text{Nd}^{3+} + \text{UO}_2^{2+}$ (—). Emission monitored at 1,055 nm.

is presented in Fig. 1. Due to the energy transfer from UO_2^{2+} to Nd^{3+} , the integrated fluorescence intensity of Nd^{3+} excited in this region is increased 8.5-fold. In the total spectral range from 360–800 nm this increase amounts to 150%. This percentage is equal to the number of photons of the solar spectrum affecting the co-doped glass divided by the number of photons affecting the Nd^{3+} -only doped glass. This ratio was calculated using the formula:

$$\frac{S_{\text{cod}}}{S_{\text{dop}}} = \frac{A^1 \times \eta_{\text{tr}} \times \eta^1 + A^2 \times \eta^2}{A^2 \times \eta^2} \times 100 \quad (1)$$

Where S_{cod} is area under the excitation spectrum of the co-doped glass, and S_{dop} the area under the excitation spectrum of the Nd^{3+} doped glass. The area of the excitation spectrum was corrected for the spectral response of the xenon source and the excitation monochromator. η_{tr} is energy transfer efficiency from UO_2^{2+} to Nd^{3+} , A^1, A^2 = absorbance of UO_2^{2+} and Nd^{3+} respectively, and η^1, η^2 = fluorescence quantum efficiency of UO_2^{2+} and Nd^{3+} respectively.

The emission spectrum of Nd^{3+} and Ho^{3+} glasses co-doped with UO_2^{2+} are presented in Fig. 2. The emission maxima correspond to the maximum sensitivity of the silicon solar cells⁷. The efficiency of energy transfer was calculated using equations (2) and (3) and is presented in Table 1.

$$\eta_{\text{tr}} = 1 - \frac{\tau_d}{\tau_d^0} \quad (2)$$

$$\eta_{\text{tr}} = 1 - \frac{I_d}{I_d^0} \quad (3)$$

Where τ_d is the first e -folding time⁶ of UO_2^{2+} in the presence of Nd^{3+} and τ_d^0 is the first e -folding time of UO_2^{2+} alone. I_d and I_d^0

Fig. 2 Fluorescence emission spectra of Nd^{3+} and Ho^{3+} co-doped with UO_2^{2+} when excited into the maximum absorption peak of UO_2^{2+} .

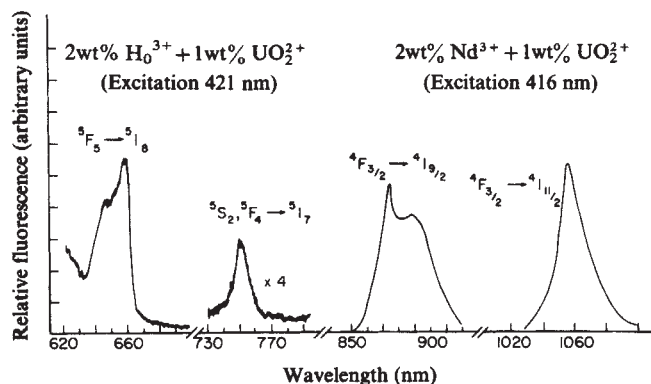


Table 1 Energy transfer efficiency between $\text{UO}_2^{2+} \rightarrow \text{Nd}^{3+}$ and $\text{UO}_2^{2+} \rightarrow \text{Ho}^{3+}$ using equations (2) and (3) (excitation at 420 nm)

Concentration (wt %)	Decay time (μs)	η_{tr} eqn (2)	η_{tr} eqn (3)
1 wt % UO_2^{2+}	205		
1 wt % UO_2^{2+} + 2 wt % Nd^{3+}	90	0.56	0.65
1 wt % UO_2^{2+} + 2 wt % Ho^{3+} *	145	0.29	0.55

* The discrepancy of η_{tr} obtained by the two methods in the case of Ho^{3+} will be discussed in a following paper.

are the fluorescence intensities of UO_2^{2+} with Nd^{3+} and UO_2^{2+} alone respectively.

From the above it can be seen that the efficiency of the previously proposed fluorescent collector can be significantly increased by utilisation of energy transfer from UO_2^{2+} to Nd^{3+} .

The practical application of Ho^{3+} co-doped glass is still doubtful because of the low branching ratio of the $^5\text{F}_4$, $^5\text{S}_2 \rightarrow ^5\text{I}_2$ and $^5\text{F}_5 \rightarrow ^5\text{I}_8$ transitions.

We thank Enrique Berman and Professor Fred Singer for their interest in this work. Patents for the convertor described here are pending.

Received 21 September; accepted 9 November 1979.

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Zirconolite—a fluorite-related superstructure

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Synthetic zirconolite, $\text{CaZrTi}_2\text{O}_7$, has attracted interest recently, as it has been proposed as a constituent phase of an artificial rock (SYNROC) which may immobilise, in solid solution, the elements occurring in high-level nuclear reactor wastes¹. Although it is known that zirconolite is a superstructure phase, derived by ordering of an anion-deficient fluorite (CaF_{2-x} or Mo_{2-x}) type of substructure², the crystal structure proper, and thus the nature of the ordering, is not known. This has hampered discussion of the crystal chemistry of the phase. A crystal structure determined from powder X-ray diffraction intensities and adequate for such discussion purposes is reported here.

Single-phase $\text{CaZrTi}_2\text{O}_7$ was made by heating the component oxides mixed in stoichiometric proportions at 1,450 °C for 4 days. Some of the product was crushed and examined in an electron microscope fitted with a tilting stage. Single crystal fragments were selected, and appropriate electron diffraction patterns were recorded. These confirmed the published crystallographic relationship of the supercell to the subcell², and enabled the space group of the supercell to be identified as either the centrosymmetric C2/c or the non-centrosymmetric Cc . Lattice parameters were determined from a powder X-ray diffraction photograph taken with a Guinier camera using

$\text{CuK}\alpha_1$ radiation and with thorium ($a = 5.5972 \text{ \AA}$) as an internal standard (Table 1).

Well crystallised zirconolite may be prepared quite readily^{2,3}, but at least one attempt to determine the structure from single-crystal X-ray diffraction data has been unsuccessful (W. G. Mumme, personal communication). It was suspected that the data may have been affected by multiple twinning in the crystal: this frequently accompanies the crystallisation of materials with a high degree of pseudosymmetry, and may not be readily detectable. Therefore, powder X-ray diffraction intensity data have been used to determine the structure. This technique has been successful for some other oxides with fluorite-related superstructures which could not be prepared as suitable single crystals.

Powder X-ray diffraction intensities for $\theta < 40^\circ$ were recorded on chart, using a powder diffractometer and filtered $\text{CuK}\alpha$ radiation. Peaks were separated where practicable, and their areas measured with a planimeter. A reflection or group of reflections was assigned to each such observation, giving a data set composed of 295 reflections distributed among 155 observations. Structure refinement was carried out using a full matrix least-squares computer program for the treatment of powder data.

As the unit cell is composed of eight fluorite M_4O_8 subcells, it has the contents $\text{Ca}_8\text{Zr}_8\text{Ti}_{16}\text{O}_{56}\square_8$ ($\square$, oxygen vacancy), an assignment confirmed by density measurement. The structure was determined by trial and error according to procedures used in similar circumstances^{4–6}. Trial structures, comprising all possible ordered arrangements of metal atoms allowed by the space groups, with atoms on the ideal fluorite positions of the supercell and all oxygen sites occupied, were refined, due care being taken in the removal of initial degeneracy. These trial structures were rejected or retained according to the value of the crystallographic residual R , which expresses the agreement between observed and calculated intensities.

It was found that one structure in C2/c (and its equivalent in Cc) was greatly superior to all the others. With this model, the site of the oxygen vacancy was found by refining the occupancies of the eight oxygen atoms in the asymmetric unit, when one fell immediately to zero, while the others were unaffected. The extent of disorder in the metal atom array was assessed by refining cation occupancies. The refinement converged satisfactorily to $R = 0.054$, with reasonable values for the refined parameters and their calculated standard deviations. An overall isotropic temperature factor was used, and on refinement, it assumed a value close to zero, with a relatively large standard deviation. As powder work involves low-angle data and non-ideal specimen surfaces, the temperature factor may have little significance as a structural parameter.

There was no indication that the structure was not centrosymmetric: refinement of the trial structure in Cc (which has 4-fold sites only) produced the same structure as that found in C2/c within calculated standard deviations of atomic parameters, and there was no significant improvement in the fit of observed and calculated intensities, despite the greater number of variable parameters. Also, a test for the absence of a

Table 1 Crystallographic data for zirconolite, $\text{CaZrTi}_2\text{O}_7$

Unit cell:
 $a = 12.4458(7)^* \text{ \AA}$, $b = 7.2734(4) \text{ \AA}$,
 $c = 11.3942(9) \text{ \AA}$, $\beta = 100.533(7)^\circ$.
 Space group: C2/c (No. 15, C_{2h}^6) (ref. 11).
 $Z = 8$. $D_x = 4.442 \text{ g cm}^{-3}$ $D_m = 4.45(5) \text{ g cm}^{-3}$
 Relationship of supercell and subcell axes:

$$\begin{pmatrix} a \\ b \\ c \end{pmatrix} = \begin{pmatrix} -1 & -1 & 2 \\ 1 & -1 & 0 \\ 3/2 & 3/2 & 1 \end{pmatrix} \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix}$$

* Numbers in parentheses are calculated standard deviations, which apply to the last quoted place.

Table 2 Structure parameters

Atom	Point set	Occupancy	x	y	z
M1	8(f)	Ca	0.3718(12)*	0.1245(19)	0.4952(9)
M2	8(f)	0.93(3)Zr +0.07Ti†	0.1225(6)	0.1222(10)	-0.0258(5)
M3	8(f)	Ti	0.2498(11)	0.1223(21)	0.7465(9)
M4	4(e)	0.86Ti +0.14Zr	$\frac{1}{2}$	0.055(2)	$\frac{1}{4}$
M5	4(e)	Ti	0	0.127(3)	$\frac{1}{4}$
X1	8(f)	0	0.310(3)	0.133(6)	0.275(3)
X2	8(f)	0	0.470(3)	0.146(5)	0.102(3)
X3	8(f)	0	0.197(4)	0.083(5)	0.573(3)
X4	8(f)	0	0.403(3)	0.174(5)	0.719(3)
X5	8(f)	0	0.702(4)	0.169(4)	0.590(3)
X6	8(f)	0	-0.001(3)	0.111(5)	0.414(3)
X7	8(f)	0	0.119(4)	0.055(4)	0.788(3)
X8	8(f)	□	$\frac{5}{32}$	$\frac{1}{8}$	$\frac{3}{16}$
Mean distances in Å					
Ti-O		1.95(7)	Ti-□		2.21(2)
Zr-O		2.19(14)	Zr-□		2.396(5)
Ca-O		2.49(7)	M-O (fluorite)		2.175
O-O		2.76(14)	O-□		2.01(9)
			O-O (fluorite)		2.512

* Numbers in parentheses are calculated standard deviations, which apply to the last quoted place.

† There is only one formal occupancy variable, because of the stoichiometry restraint.

centre of symmetry, based on the detection of nonlinear optical effects⁷, yielded a null result.

The structural parameters determined are collected in Table 2. In this structure, Ca atoms are coordinated by eight oxygen atoms at the corners of a distorted cube, Zr atoms (overlooking the minor components at mixed-occupancy sites for convenience) by oxygen at seven of the eight vertices of a distorted cube, and Ti by six oxygen atoms, either at the vertices of octahedra (M3 and M5) or a trigonal prism (M4). All these results accord with crystal-chemical expectations^{8,9}. The 'vacant' oxygen sites occur in pairs, separated by a lattice vector of the type $\frac{1}{2}$ [111] (fluorite), with a Ti atom M3 or M5 in between, and, as expected on electrostatic grounds, the relaxation of atoms from their ideal fluorite positions is such that the tetrahedron of metals (cations) surrounding a vacancy is expanded, whereas the octahedron of

oxygens around it is contracted. Similar effects have been found in all of the anion-deficient fluorite-related superstructures studied so far.

In the superstructure, there are planes of metal atoms parallel to (001) which are derived from the f.c.c. fluorite 'close-packed' metal-only (111) planes. The cation ordering is such that these planes alternately contain Ti only (A planes) and Ca+Zr (B planes). The (001) sheets of TiO₆ octahedra (Fig. 1) are similar to the sheets of octahedra parallel to {110} in the pyrochlore structure¹⁰. Within each B layer, Ca and Zr occupy alternate rows, which run parallel to [110] and $[\bar{1}\bar{1}0]$ in alternate B planes.

This structure determination provides an adequate basis for discussion of the crystal chemistry of zirconolite. However, crystals suitable for X-ray study have been produced in the CSIRO Division of Mineral Chemistry, and the structure has been determined independently from single crystal data, using direct methods. The refined results are more precise than those above, and reveal some interesting new structural effects; nonetheless, the overall agreement between the two determinations is excellent. The single crystal work has been done jointly with I. E. Grey, R. J. Hill and A. F. Reid (Mineral Chemistry) and B. M. Gatehouse (Monash University), and will be published elsewhere. Also, a structure study of non-stoichiometric zirconolite, and of zirconolite containing various oxides in solid solution has been completed and will be reported in the near future.

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OCS, stratospheric aerosols and climate

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Carbonyl sulphide (OCS) is found to be the predominant sulphur-bearing compound in our atmosphere¹⁻³. It contributes to the formation of stratospheric sulphate aerosol particles⁴, which affect the Earth's radiation balance and climate⁵⁻⁷. Using recently obtained data, we estimate that OCS has a global source of ~5 tg per year (tg = 10¹² g) and a lifetime of roughly 1 yr. We calculate that increasing anthropogenic emissions of OCS could cause measurable climate alterations within the next century. Numerous sources of OCS have been identified (see Fig. 1). Crutzen *et al.*⁸ estimate that natural and agricultural fires contribute 0.2-0.3 tg of OCS to the atmosphere each year. Adams *et al.*⁹ measured average OCS emission rates for a variety of common soils of about 0.004 g m⁻² yr⁻¹, which may be extrapolated to a global OCS source of nearly 0.5 tg yr⁻¹. Adams *et al.*⁹ also noted OCS emissions several thousand times greater than average above saline marshes. Carbonyl sulphide has been detected near cattle feedlots in concentrations as high as 6,000 p.p.b.v.¹⁰. Volcanoes and fumaroles seem to represent a minor source of OCS (refs 11, 12). We estimate that the direct contributions of biospheric processes to the OCS budget may be ~1 tg yr⁻¹.

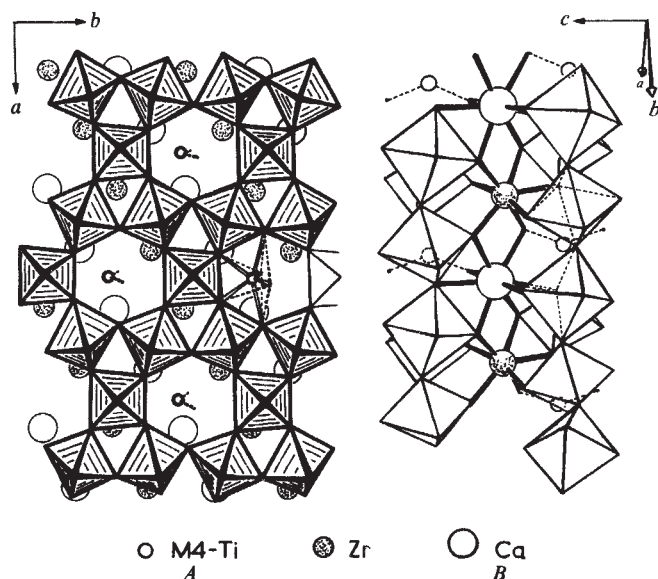


Fig. 1 A, (001) sheet of TiO₆ octahedra with parallel plane of Ca+Zr below it. The Ti atoms M4 in the sheet are associated with two O atoms X2, which are not in the octahedra: The trigonal prismatic coordination of one M4 is outlined. B, Portion of the structure viewed down [110] approximately, showing coordination of Ca and Zr.

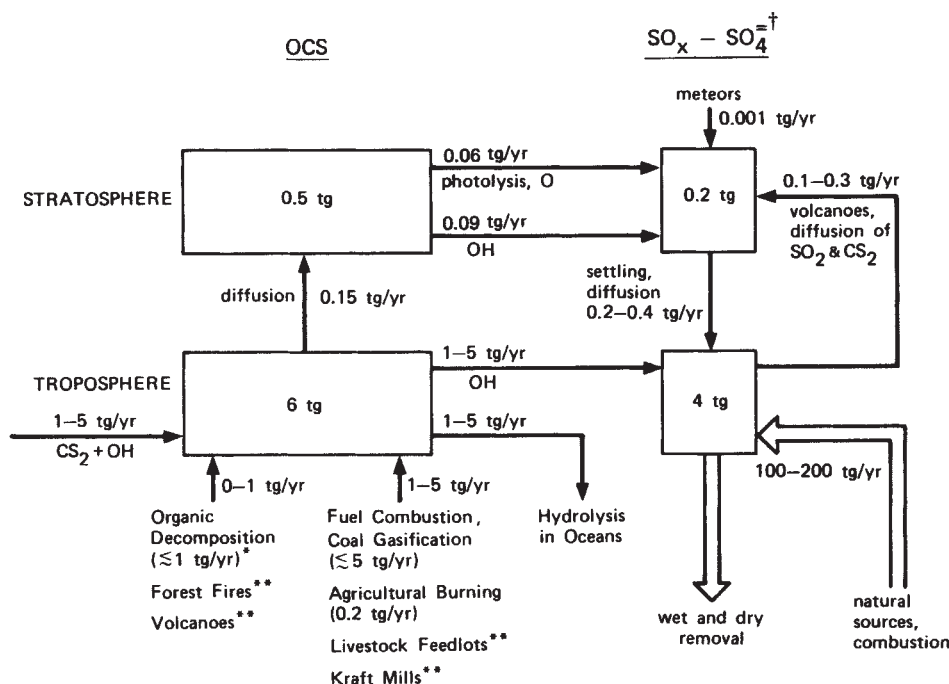
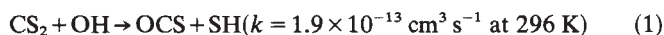


Fig. 1 Atmospheric budget of carbonyl sulphide, and of other sulphur compounds. Masses are given in teragrams (tg). (The small mass difference between OCS and SO_2 molecules is ignored.) $\dagger$ Masses are expressed in terms of an equivalent amount of SO_2 . * The source is difficult to quantify, but appears to be significant. ** The source is small, probably much less than 1 tg yr^{-1} .

Kurylo¹³ has suggested that carbon disulphide is a precursor of OCS via the reaction



Using Kurylo's rate constant and observed daytime OH concentrations¹⁴⁻¹⁷, the CS_2 lifetime against conversion into OCS appears to be $\sim 1-3$ months¹⁸. CS_2 concentrations of 0.07–0.37 p.p.b.v. have been measured by Sandalls and Penkett¹⁹ in the UK. Interestingly, these amounts are roughly consistent with the quantity of CS_2 found dissolved in the Atlantic Ocean by Lovelock²⁰ ($\sim 5 \times 10^{-13} \text{ g ml}^{-1}$) assuming equilibrium between the atmosphere and oceans. Adopting a uniform tropospheric CS_2 concentration of 0.07 p.p.b.v. (taking the lowest value observed by Sandalls and Penkett as the background abundance), an average CS_2 lifetime of two months, and unit conversion of CS_2 into OCS, we estimate that 5 tg yr^{-1} of OCS could be generated from CS_2 .

Sources of atmospheric CS_2 could include the oceans²⁰, marshes⁹ and industrial processes^{21,22}. Until more quantitative information concerning the global budget of CS_2 is gathered, however, we favour an estimate of $1-5 \text{ tg yr}^{-1}$ of OCS from the decomposition of natural and manmade CS_2 .

The refining and combustion of fossil fuels currently releases about 100–150 tg of SO_2 to the atmosphere each year. The average sulphur residue in coal is $\sim 1\%$ by mass, and in petroleum, 0.2% (refs 25, 26). Assuming that 6% of the emitted sulphur is OCS (where 6% is a maximum value for existing sulphur recovery systems²¹) the OCS combustion source could be $6-9 \text{ tg yr}^{-1}$. Of course, only a small fraction of worldwide combustion emissions are processed for sulphur removal, and untreated stack gases generally have very low OCS concentrations²¹. Hence, the OCS emission might be less than 1 tg yr^{-1} .

During coal gasification and liquification, highly reducing conditions prevail, and OCS may be produced in fractional quantities as large as 10^{-3} of the total gas volume. After scrubbing of the tail gases, however, emissions of OCS from these coal plants should (typically) amount to less than 1% of the sulphur in the coal processed²¹. Catalytic converters used in cars occasionally generate large quantities of H_2S and OCS (ref. 27). Although car petrols have very low sulphur contents—commonly 0.03–0.06% S by weight (ref. 21)—all of the sulphur is released to the atmosphere. Thus, a small contribution to the global OCS budget from petrol consumption could be expected. Negligible anthropogenic sources of OCS include direct com-

mercial production²¹, cigarette smoke²⁸, vapours from cooking grain mashes²⁹, and Kraft mills³⁰.

We conclude that the total global source of atmospheric OCS may be in the range of 1 to 10 tg yr^{-1} (see Fig. 1), and that a large fraction of this, perhaps as much as 50%, may result from anthropogenic activities related mainly to fuel processing and consumption.

Ultraviolet photolysis of OCS above 20 km is a sink for OCS and a source of sulphur for stratospheric aerosols⁴, but leads to an overall OCS atmospheric lifetime of 50 years or more. Reaction with OH may be the largest atmospheric

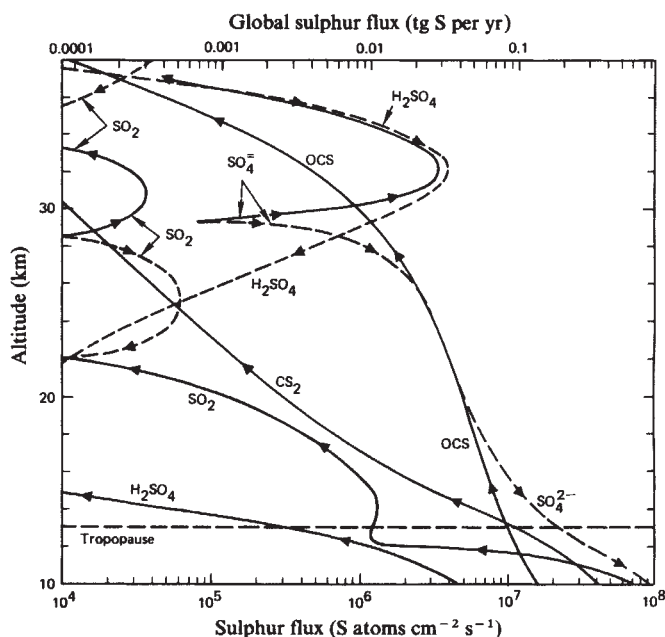
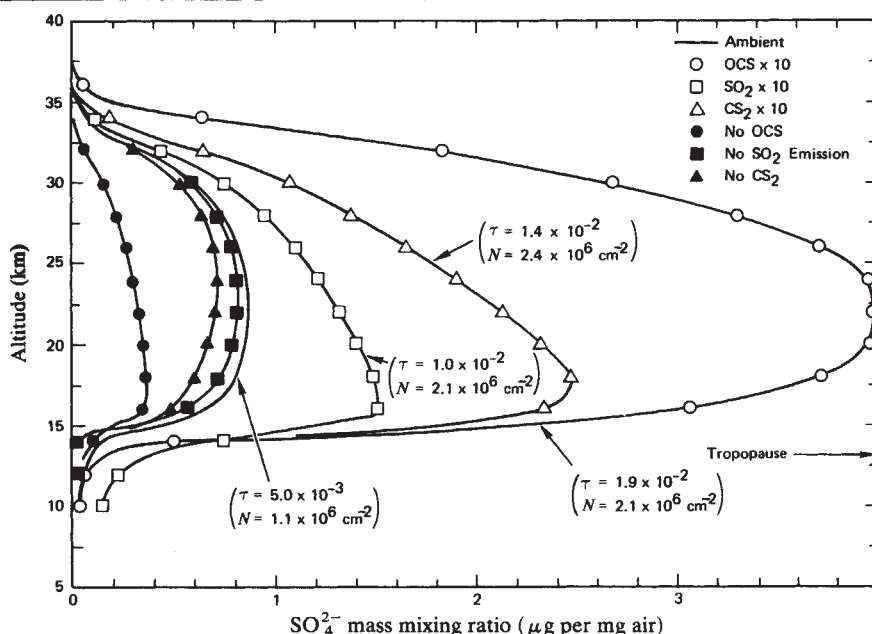
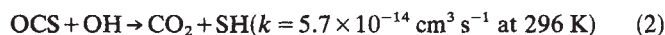


Fig. 2 The stratospheric balance of gaseous sulphur and aerosol sulphate in a one-dimensional model^{33,34}. Equivalent sulphur atom fluxes are given. The arrows indicate direction of sulphur flow (also, solid lines correspond to upward fluxes, dashed lines to downward fluxes). The SO_2 flux divergence is complicated because of SO_2 sources near 15 km due to CS_2 decomposition, 29 km due to OCS photodissociation, and 50 km due to H_2SO_4 photolysis³³. An H_2SO_4 source exists above 30 km as a result of aerosol droplet transport and evaporation³⁴.

Fig. 3 Stratospheric aerosol sulphate (SO_4^{2-}) mass mixing ratios predicted for ambient and perturbed conditions. A 10-fold enhancement in OCS, SO_2 or CS_2 is simulated by increasing its surface concentration 10-fold, resulting in roughly a factor of 10 increase in its surface emission rate and tropopause concentration, while the abundances of the other two constituents are essentially unaltered. For several of the cases treated, the total large particle ($r > 0.15 \mu\text{m}$) column concentration in the stratosphere (N) and the optical depth of the sulphate layer at 550 nm (τ) are given in brackets.



sink for OCS^{13} ,



For likely tropospheric OH concentrations¹⁴⁻¹⁷ reaction (2) implies roughly a one-year atmospheric lifetime for OCS. Reactions of OCS with ground state and excited oxygen atoms can be ignored.

Although hydrolysis of OCS is rapid in basic solutions, it is quite slow (weeks to months) in neutral²¹ and acidic³¹ solutions. OCS also has only a moderate solubility ($1.4 \text{ g l}^{-1} \text{ H}_2\text{O STP}$). Accordingly, we deduce that scavenging of OCS by clouds, aerosols and rainfall may be neglected. Seawater, by contrast, has a pH greater than 7, which implies efficient OCS hydrolytic decomposition. It has been estimated (F. S. Rowland; personal communication) that oceanic hydrolysis could lead to a global OCS lifetime as short as a year.

Carbonyl sulphide has been measured extensively in the troposphere^{1-3,19} and lower stratosphere³² and is the predominant sulphur-bearing compound in the atmosphere. OCS has a ubiquitous tropospheric concentration of about 0.5 p.p.b.v. with no apparent gradient between the boundary layer and the upper troposphere. OCS also has a small ($\sim 5\%$) interhemispheric contrast³. In addition, OCS variability (from one measurement to another) is found to be less than 10% (refs 2, 3, 19). All these observations are consistent with an OCS lifetime of the order of one year.

With reference to Fig. 1, the 6 tg of OCS measured in the atmosphere is compatible with an OCS source of 6 tg yr^{-1} and a tropospheric lifetime of one year, similar to the values we have estimated. Recent model calculations of the ambient atmospheric sulphur balance^{33,34} indicate that observed OCS, CS_2 and SO_2 abundances are roughly consonant with global surface emission rates of 3 tg yr^{-1} OCS, 4 tg yr^{-1} CS_2 and $100\text{--}150 \text{ tg yr}^{-1}$ SO_2 .

The stratospheric sulphur balance for non-volcanic conditions is shown in Fig. 2 (based on a one-dimensional model calculation³³). The dominance of OCS is clearly brought out, although CS_2 and SO_2 also contribute significantly to the stratospheric sulphur cycle. This raises the possibility that increased fossil fuel consumption rates, as have been projected by Rotty³⁵ (approximately a fourfold increase by 2025, to $23,000 \text{ tg carbon per year}$), may cause greater sulphur vapour emissions, which can alter the stratospheric sulphate aerosol, or Junge³⁶, layer. Using an appropriate model^{33,34}, we have studied the sensitivity of aerosol mass loading to changes in OCS, SO_2 and CS_2 concentrations (Fig. 3). Obviously, changes in the OCS level have the largest impact on the aerosol layer. Even so, a 10-fold

increase in CS_2 emissions could lead to a greater aerosol enhancement. Most of the CS_2 emissions might be chemically converted into OCS and SO_2 in the lower atmosphere. Hence, if CS_2 is the primary source of OCS, a CS_2 increase could produce an effect nearly as large as the combined effects of the CS_2 and OCS increases shown in Fig. 3.

The ambient aerosol layer has an optical depth of about 0.005 at 550 nm (ref. 37). For a 10-fold increase in OCS, the estimated optical depth is about 0.019. This can be compared to the optical depths observed following volcanoes Fuego (0.02–0.03) and Agung (0.02–0.04 in the Northern Hemisphere, and 0.2–0.3 in the Southern Hemisphere). Thus, a 10-fold increase in OCS produces an optical depth perturbation comparable to that of a modest volcanic eruption.

When we use these OCS optical depths in an improved version of the radiation transport model of Pollack *et al.*⁵⁻⁷, we find a possible decrease in the average global surface temperature of about 0.1 K, and a possible comparable increase in stratospheric temperatures. The surface temperature change is at the threshold of climatic significance. However, OCS, like CO_2 , can create a 'greenhouse' effect in which far IR radiation emitted by the Earth is trapped in the lower atmosphere, warming its surface and compensating somewhat the aerosol cooling.

Because of man's apparently strong influence on the atmospheric sulphur cycle, and because of the established role of sulphur emissions in air pollution episodes and possible climatic changes, we suggest that future use of fossil fuels be carefully monitored for released sulphur compounds, including OCS and CS_2 .

We thank F. S. Rowland, P. J. Crutzen and R. J. Cicerone for useful conversations on the global OCS budget. This work was supported in part by NASA grant NAS2-9881.

Received 1 October; accepted 7 November 1979.

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Low-aspect ratio ignimbrites

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Conventionally¹, three important characteristics of ignimbrites are that they show a very pronounced tendency to pond in topographic depressions, possess a flat, horizontal or gently sloping upper depositional surface, and have a thickness generally between 10 and 1,000 m. The youngest major ignimbrite, that of 1912 in the Valley of Ten Thousand Smokes (Alaska)^{2–4} shows these characteristics well, being ponded in a valley 25 km long with a flat upper surface sloping down-valley at an average of 1.3°, and having an estimated thickness exceeding 100 m in places. All three characteristics are commonly used as field criteria to distinguish ignimbrites from other pyroclastic rock bodies. Here we look at certain ignimbrites which depart significantly from this conventional form in that a major part of them occurs as a thin layer mantling the landscape, resting on slopes as steep as 30°, and with an upper surface sensibly parallel with the underlying surface. We have studied two examples, the 1,800-yr-old⁵ Taupo ignimbrite (New Zealand), and the 1,400-yr-old Rabaul ignimbrite (New Britain)^{6,7}. We also cite several others. These ignimbrites have a remarkably low aspect ratio. This ratio, previously applied to lava extrusions^{8,9}, provides a useful means of quantifying the overall geometry of rock bodies. The ratio is that of average thickness to lateral spread; conveniently the latter is taken as the diameter of the circle which covers the same area as the rock body.

The young age of the Taupo and Rabaul ignimbrites, and their occurrence in humid areas where a luxuriant vegetation anchors and protects pyroclastic deposits very soon after they have formed, has resulted in their remarkably good structural preservation. These ignimbrites shown new features which require that a reappraisal be made of emplacement mechanisms, and new diagnostic criteria be made for recognising pyroclastic types.

'Ignimbrite' is a rock body made predominantly from pumiceous material (pumice, glass shards), which may or may not be welded, and has characteristics indicating that it was emplaced

as a hot and concentrated particulate flow. By concentrated we mean that the volumetric particle:gas ratio in the moving flow was relatively high; pumice density¹⁰ and fluidisation¹¹ studies on non-welded flows indicate that they were little expanded beyond their observed density. We thus use the word ignimbrite in a genetic sense, more or less synonymously with ash flow.

The outstanding feature of the Taupo and Rabaul ignimbrites is that both occur in two contrasted forms (Fig. 1) having quite different relationships to the pre-existing land surface: one form occurs as a mantling layer generally less than 2 m thick which more or less completely drapes the landscape; the other form occurs in the 'conventional' way as valley-ponds having a nearly horizontal upper surface and locally attaining a depth exceeding 10 m. The name 'ignimbrite veneer deposit' is proposed for the landscape-blanketing form which connects the valley-ponds in a continuous sheet.

At the edge of the valleys the ignimbrite veneer deposit is visibly continuous with the ponded ignimbrite, and this continuity is observed at so many points that there is no doubt that both forms are part of a continuous rock body. The ignimbrite veneer deposit has practically identical grain size and constitution to the ponded ignimbrite, except that it lacks the coarsest pumice clasts, and it shows a crude layering in which the layers are 1–20 cm thick and differ from one another mainly in maximum pumice size. Locally it also contains discontinuous lenses of well rounded and well sorted pumice.

Our interpretation of the veneer deposit is that the pyroclastic flow surmounted more or less the entire landscape when spreading outwards from source, and after the passage of the main body of the flow the material drained only very incompletely into the valleys, leaving behind a mantling layer on the interfluvies. This layer lacks the coarser pumice which, being concentrated in the upper or middle parts, moved on with the body of the flow. The veneer is a 'tail' deposit left in the wake of the pyroclastic flow.

The significance of an ignimbrite veneer deposit is that it is deposited from, and records the passage of, a pyroclastic flow. In the Taupo example, its presence shows that the flow surmounted hills rising more than 1,000 m above the present level of the vent.

The closest analogue to the Taupo and Rabaul ignimbrites in the literature is the Koya flow (Japan)¹² which extends to 60 km from the vent with a thickness of generally less than 1 m. The Lajes ignimbrite in Terceira (Azores)¹³ reaches a thickness of 10 m at several places around the coast of the island which are interconnected by a thin layer, now interpreted as a veneer deposit, draped over the intervening country. An example of a densely welded ignimbrite is known on Fantale (Ethiopia)¹⁴ where it clearly occurs as two forms, one as a layer 1–2 m thick spread over much of the surface of the volcano, including some upstanding lava domes, and the other ponded at the foot of the volcano. The continuity of the densely welded Skessa tuff of Tertiary age in Iceland¹⁵, which extends over 430 km² with an average thickness of 6 m, appears to have more the characteristics of a veneer deposit than those of a 'conventional' ignimbrite.

Recognition of the ignimbrite veneer deposit as a distinct type requires that the diagnostic criteria for the various pyroclastic types be updated. Similarly to a pyroclastic fall deposit, it mantles the topography and has internal layering. However, it is distinguished by the rapid lateral variations in thickness and occasional cross-bedding shown by the layers, and by the local presence of laterally discontinuous pumice lenses. Like a base surge deposit¹⁶ it shows a distinct cross-bedding, but the angle between layers is commonly less than 10°, much less than in surge deposits. Moreover, the grain-size contrast between layers is negligible compared with that in surge deposits and it also contains carbonised vegetation.

The extensive ignimbrite literature concentrates on the larger-volume and more impressive examples, those which are generally in part at least welded and have smothered the existing landscape to create their own new aggradational surface.

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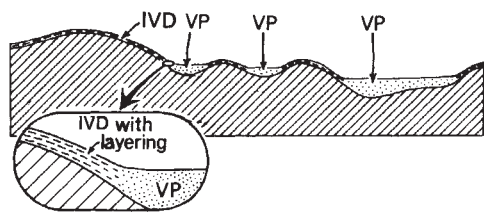


Fig. 1 Sketch showing the relationship between the landscape-mantling ignimbrite veneer deposit (IVD) and the associated valley-ponded ignimbrite (VP) as exhibited by the Taupo and Rabaul ignimbrites.

Workers in volcanic areas have therefore tended to regard the features of these big ignimbrites as characteristic of all ignimbrites; there is a reluctance to apply 'ignimbrite' or its synonym 'ash flow' to thin non-welded sheets, particularly when they mantle the topography and display a crude layering.

The word ignimbrite was originally applied to welded ash flows¹⁷, but it has since become clear that welding of an ignimbrite is largely an accident of circumstance; it depends for example on the eruptive column height (as this determines the magnitude of the heat loss at the vent¹⁸) and on the thickness of the sheet. If 'ignimbrite' is applied in a genetic sense to rock bodies which have been emplaced by a common pyroclastic flow mechanism, then its definition should include non-welded as well as welded examples. Similarly, thin and thick, landscape-mantling and valley-ponded, variants should also be included.

The veneer deposit of the Taupo ignimbrite covers about 15,000 km² with an average thickness of ~1 m, and it contains roughly half of the total volume of the ignimbrite. The veneer deposit of the Rabaul ignimbrite covers well over 1,200 km²—we did not attempt to trace it to its extremities—with an average thickness of ~2 m, and constitutes probably more than half of the total volume. The description of the Koya flow suggests that it probably consists entirely of a veneer deposit. Even the Valley of Ten Thousand Smokes ignimbrite shows a 'high sand mark'¹⁹ which is especially well developed in the near-vent area and described as reaching 60 m above the adjacent valley-pond level towards which it slopes at 10–15°, and may be interpreted as a veneer deposit. We propose that ignimbrites form a spectrum of types, ranging from those in which the veneer deposit constitutes the entire volume, to those in which it constitutes none.

Ignimbrites such as Taupo, Rabaul (and Koya?), in which the veneer deposit constitutes a large proportion of the total volume, may be characterised as ignimbrites of low aspect ratio type. These three examples have an aspect ratio of 1:70,000, 1:7000 and ~1:200,000 respectively, and the Skessa Tuff 1:4,000. In contrast, the VTTS ignimbrite has an aspect ratio of about 1:400 and the Bishop Tuff²⁰ about 1:250.

It remains to consider why these ignimbrites have a low aspect ratio. It is known that the Taupo ignimbrite eruption was immediately preceded by a plinian phase in which the eruptive column, judged from the wide dispersal of the plinian pumice, was quite exceptionally high²¹, and the low aspect ratio is attributed to the high momentum imparted to the pyroclastic flow when catastrophic collapse from this high column occurred and also to the high magma discharge rate. There is good evidence that the Rabaul column was similarly very high. We lack detailed information on the Koya event. In all three ignimbrites the density of the pumice is unusually low, but we think that this is more significant to the dynamics of the explosive eruption than to the mobility of the pyroclastic flows.

G.P.L.W. is a Captain James Cook Research Fellow of the Royal Society of New Zealand, and C.J.N.W. a NERC research student; fieldwork of R.F. was supported by the Research Fund of Auckland University.

Received 4 September; accepted 5 November 1979.

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Exploring pictures tactually

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Raised-line drawings of common objects can be identified by congenitally and early blind subjects^{1,2} and displays of this type are being devised for use in education (for example, ref. 3). Although a teacher may think it helpful to guide a blind student's hand around one of these drawings, several theories of perception predict that the guidance would be detrimental as passive perception is generally considered to be inferior to active perception (for review see ref. 4). We show here, however, that guidance is helpful and leads to better identification than unguided exploration. We also report that the information used to identify raised-line drawings is predominantly kinaesthetic.

Our first two studies compared active and passive conditions for recognition of raised-line drawings. Although the practical application of this comparison concerns the blind, the issue can be tested using blindfolded subjects. In the first study, eight raised-line drawings of familiar objects were presented individually to 40 blindfolded students. Half of the subjects were passive, that is, the experimenter traced the index finger of the subject's preferred hand around a figure, controlling the direction, extent and speed of movement. The performance of these passive subjects excelled that of the active subjects, who determined their own exploration. The passive group successfully identified 42 drawings compared with 20 identified by the active group, a highly significant difference according to a ranked sums test ($P < 0.005$). This study has the practical implication that a blind student could profit from the guidance of a sighted instructor when first trying to identify raised-line drawings.

Our unexpected result prompted us to carry out a yoked comparison of the two types of exploration in which the movements of a passive subject's finger were matched moment by moment to those of an active subject. Yoking was accomplished with the use of a computer system and a digitised tablet on which the drawing rested. The position of a small coil surrounding the index finger of a subject's preferred hand was monitored within the area of the tablet to the nearest 0.25 mm and recorded every 200 ms to retain a detailed record of exploratory behaviour. The record of the path traced by an active subject could be 'played-back' on a television monitor situated next to the digitised tablet. The entire path was outlined by dots, each dot specifying the location of the centre of the coil at a particular time. A small cursor moved to each dot in turn, thus retracing the subject's exploratory movements.

Eight naive subjects participated as active explorers. One of 12 raised-line drawings was taped to the tablet surface as each blindfolded subject individually explored the complete set of drawings. Each subject was instructed to trace the figure in a clockwise direction, without repetition (retracing). A complete set of tracings was recorded for each of the active subjects exploring each of the 12 drawings presented in different random orders. As an example, Fig. 1 shows a copy of a tracing by an active subject.

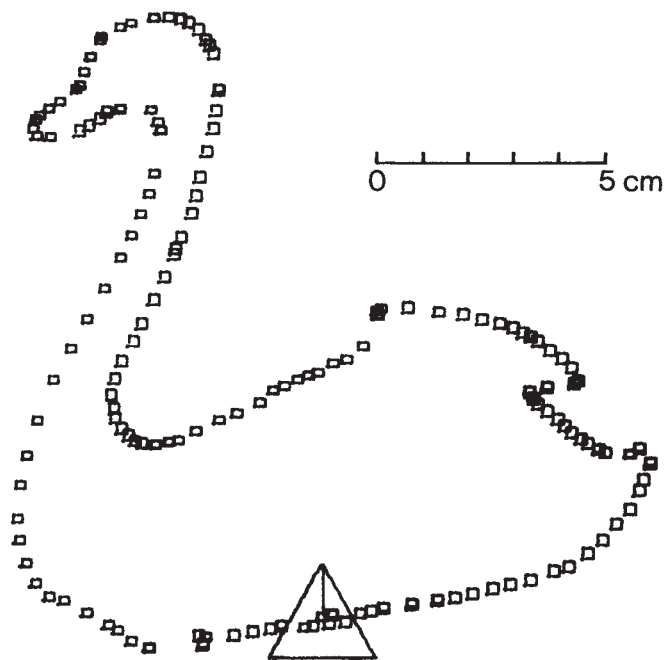


Fig. 1 A tracing of a raised-line drawing of a swan by an active subject. Each small square represents the position of the subject's finger at 200-ms intervals. Exploration began at the centre of the large triangle (not actually present as a raised-line) and proceeded in a clockwise direction. The density of squares therefore indicates the speed at which the finger was moved over each part of the display. An analogue of this figure was available on a monitor to guide the experimenter when passive subjects participated in exploring the form. All raised-line drawings for this experiment were formed by a single continuous line defining the outline of an object easily identifiable by sight. These included a drawing of a butterfly, a boat, a crown, a fish, a goblet, a claw hammer, a hand, a body, a rabbit, an umbrella, a swan and a flower.

Eight passive subjects encountered each of the drawings as presented to their active counterparts. At the same time as the recording of an active subject's performance was replayed by the monitor, the experimenter traced the passive subject's finger along the drawing. A second cursor on the monitor showed the current location of the passive subject's finger. The experimenter, watching the monitor, kept the passive subject's cursor as close as possible to the replay.

A record of the passive subject's finger locations was kept so that the degree to which the active and passive tracings departed could be determined. This involved a calculation of the distance between the active and passive subjects' positions for each interval averaged over the number of intervals needed to complete the trace. As the cursor for the passive subject normally followed the cursor for the active subject, mean deviation was computed for various time lags and a minimum value for mean absolute deviation was specified for each drawing. Averaged over the set of drawings and across pairs of subjects, the overall mean deviation was found to be 2.28 mm, at an average lag of approximately 161 ms. This value represents an average of only 0.67% difference in the areas of the two patterns. Therefore, a difference in identification rates between the two groups is unlikely to be accounted for by a difference in exposure. In agreement with the previous study, passive subjects performed better than active ones. The passive group identified 25 drawings, whereas 16 were identified by the active group, a difference significant under a Wilcoxon matched-pair signed-rank test ($P < 0.05$).

The success of passive participation readily allowed determination of the source of information subserving identification. This involved a comparison of two more groups. For one of these groups ($n = 10$), the subject's index finger was held stationary while the raised-line drawing was moved underneath the finger-

tip so that the twists and turns of the raised-line could be detected cutaneously unaccompanied by any movement on the subject's part. For a second group ($n = 12$), exposure to the raised-line drawings allowed for kinaesthesia but not for cutaneous input. Here, the subject's finger was moved passively around the outline of a figure where the raised line was not present. The experimenter simply used a clear plastic sheet superimposed on a pencil drawing for guidance. Only five figures were successfully identified by the 'cutaneous' group, whereas 25 figures were identified by the 'kinaesthetic' group. This difference is highly reliable according to a Mann-Whitney U -test ($P < 0.001$). Clearly, the ability to identify raised-line drawings does not depend on cutaneous information as it would in braille reading. It seems that information arising from limb position and limb movement is predominant. This does not mean that the raised line fails to serve a useful purpose. Obviously, the raised line is needed to guide movement during active exploration but it is on the resultant kinaesthetic information that identification of the pattern rests.

The importance of kinaesthesia suggests that the difference between active and passive performance may be accounted for by considering how initiation, execution or monitoring of movements differs in these conditions. Active subjects have the additional requirement that they must plan exploratory movements. Some of these intended movements will be translated into activity whereas others may fail to be initiated or be only partially executed. The active group may find it difficult, in memory, to separate intended movements which are acted on from those which are not. Further, the act of planning movements may draw on the limited resources of the haptic system so that less processing capacity is available to monitor and distinguish relevant from irrelevant kinaesthetic input.

We thank our colleagues at Scarborough College, University of Toronto, for valuable comments. This research was supported in part by the NRC of Canada.

Received 30 July; accepted 6 November 1979.

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Influence of brood hosts on host preferences of bark beetle parasites

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The southern pine beetle, *Dendroctonus frontalis* Zimmerman (SPB) is attacked by several species of hymenopterous parasites^{1,2} the most common of which also parasitise other bark beetles³⁻⁵ including the engraver beetles *Ips grandicollis* (Eichhoff), *I. avulsus* (Eichhoff), *I. calligraphus* (Germar), *I. pini* (Say); and the eastern juniper bark beetle, *Phloeosinus dentatus* (Say). The increasing importance of pest management has focused attention on the role of their natural enemies. However, the dynamics of bark beetle parasite populations is not generally known beyond estimates of mortality caused by parasites. The hymenopterous parasite guild of *D. frontalis* may kill up to 30% of a given brood². Since *D. frontalis* is cyclic in most areas with epidemics followed by very low population

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densities¹, other beetle hosts probably act as reservoirs for certain species of these parasites³. Conversely, during *D. frontalis* epidemics, other beetles, such as *I. grandicollis* and *P. dentatus* may compete as alternate hosts. Information on preferences for *D. frontalis* and other associated bark beetles is necessary to understand the interactions of this host-parasite complex. We report here the results of a study designed to determine if several known *D. frontalis* parasites display any host preferences among *D. frontalis*, *I. grandicollis* or *P. dentatus*.

Infestations of *D. frontalis*, *I. grandicollis* and *P. dentatus* were located in Camden, Clarke and Oglethorpe Counties, Georgia. Bolts, ~0.5 m long and 20 cm in diameter, that were uniformly infested predominantly with late instar larvae, were cut as trap bolts. Camors and Payne⁶ showed that *Heydenia unica* Cook and Davis prefers third instar larvae of *D. frontalis*, and many other parasites have similar preferences (Berisford *et al.* unpublished data). Lengths of the trap bolts were adjusted according to diameter, to maintain approximately 3,000 cm² of bark surface. Each trap bolt was infested with only one species of bark beetle. *D. frontalis* and *I. grandicollis* trap bolts were cut from loblolly pine (*Pinus taeda* L.) and *P. dentatus* trap bolts were from eastern red cedar (*Juniperus virginiana* L.). Concurrent with the above collections, bolts of similar size which had beetle pupae or callow adults with parasites that were about to emerge were cut as a source of the parasites.

All the bolts were removed to University of Georgia School of Forest Resources Whitehall experimental forest near Athens, Georgia. Traps constructed from 30 × 20 cm aluminium metal screening (16 × 18 mesh) were affixed to the trap bolts which contained third instar larvae and the screens were coated with a thin layer of 'Stikem Special'. The trap bolts were then suspended by hooks from wooden frames inside 6 × 6 × 6 ft (1.83 m) saran screen cages (32 × 32 mesh). The cages were erected under a light pine overstory. Source bolts were placed at the bottom of the cages. Sunlight was blocked from the cages by a sheet of 100 µm thick black plastic draped over the top. This prevented the parasites from orientating to the light at the top of the cages. Four trap bolts of each beetle species involved in the experi-

ment, and four source bolts, all infested with the same beetle species were used in each cage. Uninfested control bolts were used in preliminary experiments but discontinued when they were found to be unattractive to parasites. All possible two- and three-host combinations were tested except *P. dentatus* and *I. grandicollis* alone.

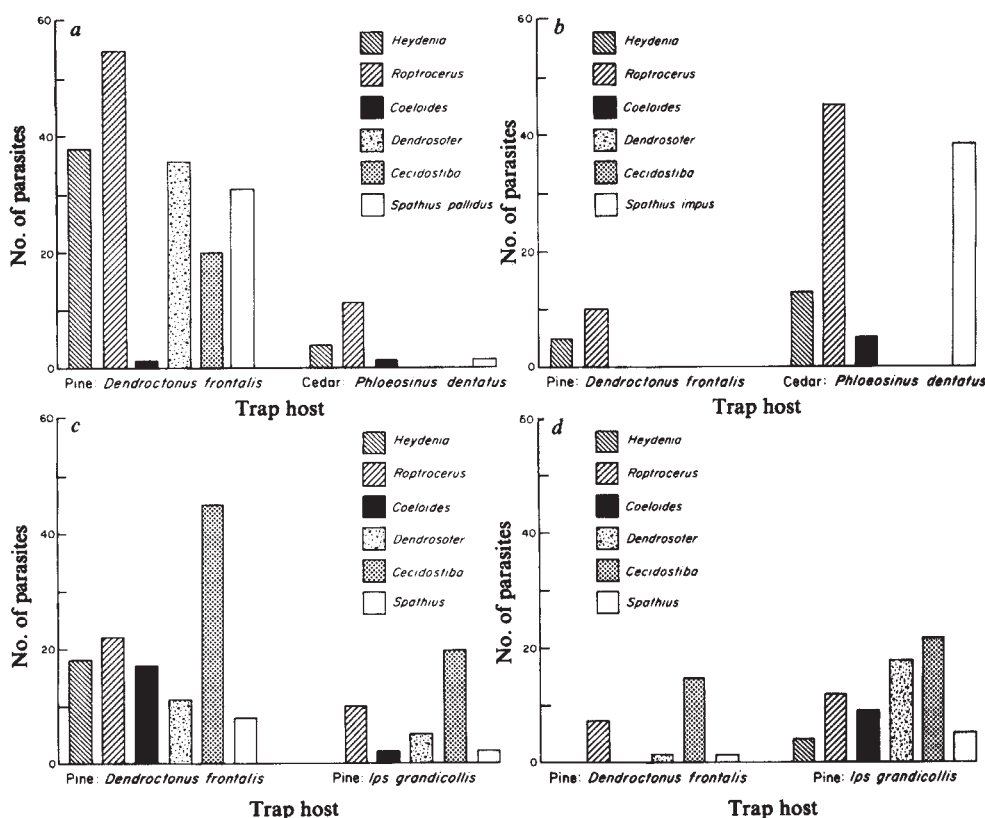
The sticky traps on the infested bolts were inspected daily for up to 2 weeks depending on parasite emergence from the source bolts. Parasites were removed from traps, cleaned in petrol and saved for identification. Trap bolts were checked as long as parasites emerged from source bolts or until larvae in trap bolts began to pupate. Responses of parasites to beetle hosts from which they were reared was compared using the normal approximation to the binomial in a one-tailed test.

During 25 field cage experiments, 681 parasites comprising six species were trapped. Parasites trapped included *Heydenia unica* Cook and Davis, *Roptrocercus xylophagorum* (Ratzeburg), *Coeloides pissodis* (Ashmead), *Dendrosoter sulcatus* Muesebeck, *Cecidostiba dendroctoni* Ashmead, *Spathius pallidus* Ashmead and *Spathius impus* Matthews.

Some of the parasites are somewhat host-specific but were included for comparison. They were not considered in the overall analysis. *S. pallidus* parasitises *I. grandicollis*⁵ and *D. frontalis*² but not *P. dentatus*. However, *P. dentatus* is parasitised by *S. impus*⁷ which is apparently host specific to *Phloeosinus* spp., *D. sulcatus*, although a frequent parasite of *Ips grandicollis* and *D. frontalis*⁵ probably does not parasitise *P. dentatus*. The orientation of these host-specific parasites to their proper host is indicative of the efficacy of the experimental technique. *Cecidostiba dendroctoni* has been suspected of parasitising *I. grandicollis*⁸. All *Cecidostiba* reared from *I. grandicollis* came from trees removed from the same site in Camden County, Georgia. Apparently, in that area, *C. dendroctoni* is a relatively common parasite of *I. grandicollis*. *H. unica*, *R. xylophagorum* and *C. pissodis* attack all three host beetles^{2-5,9,10}.

Parasites which emerged from a given host returned to that host in significantly greater numbers (Fig. 1). Parasites reared from *D. frontalis* and exposed to *P. dentatus* and *D. frontalis* as

Fig. 1 Numbers of hymenopterous parasites collected from trap bolts in field-cage experiments. *a*, Parasites reared from *Dendroctonus frontalis* and trapped on logs containing late instar larvae of *D. frontalis* and *Phloeosinus dentatus* during simultaneous exposure to the parasites. *b*, Parasites reared from *Phloeosinus dentatus* and trapped on logs containing late instar larvae of *P. dentatus* and *D. frontalis* during simultaneous exposure to the parasites. *c*, Parasites reared from *Dendroctonus frontalis* and trapped on logs containing late instar larvae of *D. frontalis* and *I. grandicollis* during simultaneous exposure to the parasites. *d*, Parasites reared from *Ips grandicollis* and trapped on logs containing late instar larvae of *I. grandicollis* and *D. frontalis* during simultaneous exposure to the parasites.



hosts showed an almost tenfold preference for *D. frontalis* (Fig. 1a). When parasites reared from *I. grandicollis* or *D. frontalis* were given a choice of these two pine beetles, the split was not so pronounced, with about three times as many parasites returning to the 'parent' host (Fig. 1c and d). This difference is probably due, in part, to parasites orienting to the tree host (pine vs cedar). *Ips* spp. parasites may manifest differential responses to different tree hosts¹¹, but both *I. grandicollis* and *D. frontalis* were in loblolly pine in our study. Differences in response in this case probably could not be attributed to the host tree.

Parasite totals for each combination of hosts were shown to be significant at the 5% level except the three host combination with parasites reared from *Ips*. Although statistically non-significant, probably due to the relatively small sample, more parasites selected bolts infested with *I. grandicollis*. These data were significant (5% level), however, when combined with the two host combinations (*Ips* and *Dendroctonus*, with parasites reared from *Ips*). Most counts for individual parasite species from each combination also showed significantly stronger attraction to the species of host from which they were reared.

This host preference phenomenon is an apparent example of Hopkins' host selection principle¹². Although the principle was proposed for phytophagous insects and has been demonstrated^{13,14}, exceptions have been noted^{15,16}. Our study is one of few apparent demonstrations of Hopkins' host selection principle involving parasites of animals^{14,17} and the first for parasites of bark beetles.

Parasites which are not host specific tend to attack the host on which they were reared but they retain the ability to use other hosts if the preferred host is unavailable. This behaviour is advantageous to the parasites in that they may use all potential hosts more efficiently by switching to relatively more abundant alternate hosts when populations of preferred hosts decrease. This phenomenon may also increase prey diversity¹⁸. The switching mechanism should be particularly helpful to species with relatively low host-finding efficiencies. The most common parasite in our study, *R. xylophagorum*, is unable to locate potential hosts in low numbers proportional to the number located when hosts are abundant³ but its ability to attack a variety of hosts apparently allows it to maintain substantial populations.

The impact of the parasite guild of the southern pine beetle may be affected by numbers of alternate hosts. Parasites may switch from other hosts during expansions of *D. frontalis* populations if *D. frontalis* becomes much more abundant than the 'preferred' host. Therefore, the host selection mechanism serves to concentrate the parasites on the epidemic host after a period of time. As the epidemic populations decline, the parasites can accept other hosts as they become relatively abundant, thereby reducing the impact of parasites on declining host populations.

We thank Drs. M. C. Birch, H. M. Kulman and F. M. Stephen for help with this manuscript and Dr. G. O. Ware for statistical expertise. These studies were supported by the USDA programme entitled 'The Expanded Southern Pine Beetle Research and Applications Program' through GAES-USFS Cooperative Agreement 18-475.

Received 6 July; accepted 9 November 1979.

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Mate choice increases a component of offspring fitness in fruit flies

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The authors of two influential accounts of the genetic consequences of mate choice consider that animals cannot produce fitter offspring by mating with a fitter than average individual^{1,2}. Their main reason for this is that in a population at genetic equilibrium any genetic variation which affects fitness should not be heritable³. Other accounts have suggested ways in which mate choice might improve offspring fitness⁴. Data are now presented which show that one component of offspring fitness can be increased by mate choice in *Drosophila melanogaster*. The component of fitness measured was intraspecific competitive success during the part of the life history from first instar larva to adult fly.

The flies used were an outbred stock collected in Dahomey in 1970 and maintained in approximately constant conditions in a population cage at the Department of Genetics, Edinburgh University. Two different experimental comparisons were made. In the first (experiment 1), the offspring of inseminated adult females which had mated in the population cage where many males were present were compared with offspring of females collected from the cage as virgins and mated with a randomly chosen male from the cage. The comparison was made by taking first instar larvae from eggs laid by the 'choice' and the 'no choice' females and competing each set of larvae against an equal number of larvae of Dahomey stock made homozygous for a fourth chromosome bearing the recessive mutant *sparkling*.

Table 1 The competitive success of offspring of flies which had chosen (choice) or not chosen (no choice) their mates

		% Wild type		No. of vials
		Mean	Variance	
First replicate	Choice	51.1	7.1	23
	No choice	48.9	8.7	18
	$t = 2.45$	$P < 0.02$		
Second replicate	Choice	49.8	2.2	21
	No choice	48.1	5.8	14
	$t = 2.45$	$P < 0.02$		

Experiment 1: The experimental details were as follows. Adult male and inseminated female flies were collected from the population cage using a pooter. After anaesthetisation with carbon dioxide, 100 males and 100 females were taken and placed individually in shell vials with food. Eclosing virgin females (100) were then collected from bottles in the population cage and placed individually in the 100 vials containing single males. After 2 days the 'no choice' females were separated from the males, and females of both groups were then set up in batches of 10 and allowed to lay eggs on 10% agar medium with a small amount of yeast present. At the same time, other batches of 10 females were set up using the Dahomey *sparkling* stock. All females were allowed to lay for 24 h before being moved on to fresh agar, and after a further 16 h first instar larvae were collected from the surface of the original agar using a paintbrush. The larvae were then placed in two groups of vials. Vials in the first group each contained 200 larvae from the 'choice' females and 200 *sparkling* larvae; vials in the second group each contained 200 larvae from the 'no choice' females together with 200 *sparkling* larvae. The vials were set up in this way over a period of 10 days, repeatedly taking offspring from the same females which used stored sperm during this period. It was therefore not known how many larvae each female contributed to each vial. The 400 larvae in each vial were allowed to compete and pupate, and the number of wild-type and *sparkling* flies emerging from each vial was recorded. Competitive success was measured as the percentage of wild-type (as opposed to *sparkling*) flies emerging from each vial. Significance levels are on a two-tailed *t*-test.

Table 2 The competitive success of offspring of females of the same age which had chosen (choice) or not chosen (no choice) their mates

	% Wild type		No. of vials
	Mean	Variance	
Choice	50.45	2.0	28
No choice	49.44	2.0	32
$t = 2.72$	$P < 0.01$		

Experiment 2: Adult flies were collected from the population cage using a pooter; after anaesthetisation with carbon dioxide the females were discarded, 100 males were placed individually in shell vials with food and 300 were placed in a single bottle with food. Eclosing females were then collected from bottles in the cage, and 100 were placed in the bottle containing 300 males and 100 individually in the 100 vials containing single males. The flies were left for 2 days, after which laying pots and competition vials were set up as in experiment 1. Competitive success was measured as the percentage of wild-type flies emerging in each vial. Probability level is on a two-tailed t -test.

Larvae were allowed to compete in vials which contained the same food medium as that used in the population cage, and the number of wild-type and *sparkling* flies emerging from each vial was recorded. The point of this experimental procedure was to ensure that the wild-type parent flies were exactly matched for nutrition and numbers of anaesthetisations, and that the larvae were competing under circumstances similar to those encountered by larvae in the population cage, both with respect to the nature of the food and the genotype of the competitors. The experiment was done twice, and the results, shown in Table 1, indicate that in both replicates the percentage of wild-type flies emerging was slightly, but significantly, higher when the parents of those flies had chosen their mates.

Because the 'choice' flies in experiment 1 were collected at an older age than the 'no choice' females, experiment 2 was carried out to control for any effect of maternal age. First instar larvae were collected from parents of the same age who had either chosen or not chosen their mates, and the larvae were again competed against *sparkling* larvae. The results are shown in Table 2. Again, the percentage of wild-type flies emerging was larger if the parents of those flies had chosen their mates.

The results show that matings between randomly chosen pairs of flies produce offspring with lower survival between larvae and adult than matings where mate choice can occur. The results do not show whether the overall fitness of the offspring is affected, because some other component of fitness may be negatively correlated with the one measured.

The result could be produced by various genetic mechanisms. First, fitness may indeed be heritable, possibly as a consequence of mutational load⁵. If this were the case, the results could be produced in two ways. (1) Fitter flies may be better at detecting or obtaining access to mates. Members of the other sex could then mate with a fly that was successful in some sort of competition with members of its own sex. (2) Flies may be able to detect heritable fitness in members of the other sex. Fitter flies could then be actively chosen as mates. Both possibilities would suggest that all flies should prefer the same sorts of mates. Alternatively, flies with high levels of heterozygosity may have high fitness. In this case, flies might produce fitter offspring by mating with individuals genetically unlike themselves, so that their offspring will have higher levels of heterozygosity. This last possibility would imply that genetically dissimilar flies would show different patterns of mate choice.

I thank Drs N. P. Ashmole, A. Ewing and L. Nunney and Professor A. Robertson for helpful advice.

Received 5 July; accepted 8 November 1979.

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Larval competition and brood sex ratios in the gregarious parasitoid *Pachysomoides stupidus*

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The haplodiploid genetics of the Hymenoptera, by which males and females develop from unfertilised and fertilised eggs, respectively, give rise to certain asymmetrical coefficients of relatedness among family members^{1–3}. These asymmetries, and predictions concerning the sex ratio that are based on them^{2,4,5}, make the Hymenoptera ideal for the study of social behaviour. Work has focused on the more highly social groups of ants, bees and wasps^{6–10}. Although there has been considerable work on the parasitoid Hymenoptera with respect to sex ratio theory^{11–15}, no study has examined the social interactions of gregarious parasitoids in the light of inclusive fitness theory¹. I discuss here conflict among family members of a parasitoid wasp over the allocation of host resources, and argue that males are the more altruistic/less selfish sex. This provides a further explanation for hymenopteran males being generally smaller than females and for investment ratios being biased towards females^{4,16}. Conflict is predicted between mothers and their daughters over the allocation of resources to the sexes. The observed brood sex ratios are interpreted as a means by which egg-layers equalise investment in the sexes¹⁷.

Pachysomoides stupidus (Hymenoptera, Ichneumonidae) is a parasitoid of the social wasp *Polistes canadensis* (Hymenoptera, Vespidae). A female alights on a host nest and lays her eggs within a cell containing an early-stage pupa. Up to 31 parasitoids develop together on a host pupa. The larvae feed externally, starting with the pupa's abdomen and finishing with its head. Before a host is consumed, some larvae may be excluded by their siblings from feeding. The wall of the cell surrounding the host constrains the larvae. When the larvae are small there is room for all of them to feed. As they move forward, when their girths fill the cross-sectional area of the cell, one larva loses access to the host while the others continue to feed. In larger groups this process is repeated. For example, at pupation in broods of 13, only six or seven are near the host's remains. The individuals farther from the remains are generally smaller.

Between August and October 1977, 56 *P. canadensis* nests with pupae were collected around Barro Colorado Island in Panama. The 70 broods of *P. stupidus* were allowed to develop in the laboratory. They came from 26 nests. Whenever possible the sex and position of brood members were recorded. Complete sex ratios were obtained for 50 broods. In the other broods 46 individuals were not sexed because of larval death, eclosion before nest capture, escape or hyperparasitism. The aggregate sex ratio for all 70 broods was close to 1:1 (292 males:308 females).

Dry weight was used as a measure of size. Males, on average, were smaller than females (means of 2.16 and 2.76 mg, respectively); however, the modal size of both sexes was equal (2.2 mg). Ranges were 0.4–5.0 mg for males and 0.5–5.7 mg for females. The large, 14-fold range in size reflects among and within brood differences. Smaller individuals came from larger broods with, on average, less food per individual. In any given brood the males were generally smaller than the females and usually pupated at the end farthest from the host's remains. Of

42 non-unisexual broods that were weighed, the heaviest individual was female in 37 and male in three; two contained a male and female of equal weight. The smallest individual was male in 33 and female in nine. Of the viable individuals for which position was known, 41 males and 155 females pupated at the host's head-end of the cell, and 95 males and 28 females at the abdomen-end.

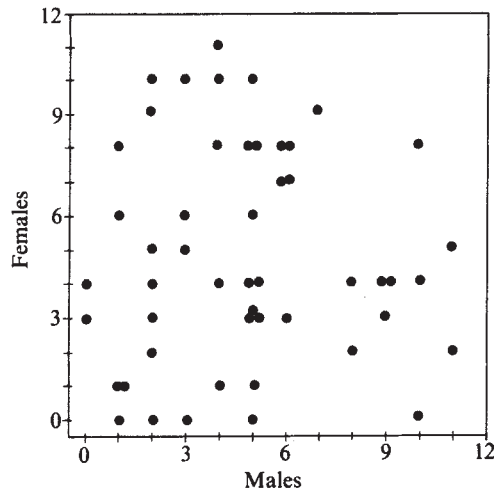


Fig. 1 Number of males and females in broods of *Pachysomoides stupidus*. The individuals from 50 complete broods reared in 1977 were sexed. Brood size ranges between 1 (a single male) and 18 (10 males and eight females), with a mean of 9.5 and a mode of 13. There are 238 males and 239 females in this sample. The distribution of sex ratios within broods was tested for overdispersion by using the statistic $D = \sum |M - F|$, where M is the number of males and F is the number of females within a brood, and the summation is over all broods. The sampling distribution of D was obtained by simulation. Males and females were assigned with equal and independent probabilities to 50 artificial broods of the sizes observed in the real sample, and the corresponding value of D was computed. This procedure was repeated 10,000 times. The resulting distribution of D values ranged between 75 and 171, with a mean of 119 and a standard deviation of 13.2, and was slightly skewed right. The value of D corresponding to the 50 real broods represented is 177. Thus the observed brood sex ratios are significantly overdispersed ($P < 0.0001$).

This competition is interesting in the light of inclusive fitness theory¹ because asymmetrically related brothers and sisters were developing where access to food by some siblings could be blocked by others. With haplodiploidy, where females develop from fertilised eggs and males from unfertilised eggs, asymmetries in the coefficients of relatedness (b) arise^{2,3}. Assuming no inbreeding, a male is more related to his sister ($b = 1/2$) than she is to him ($b = 1/4$). Sisters are related to each other by more ($b = 3/4$) than are brothers ($b = 1/2$). A mother is equally related to sons and daughters by $b = 1/2$. That males are usually smaller than females when food is limited, and yet that males are nearly as large as females if given adequate food, is explained in part by the asymmetry in relatedness between brother and sister. If the cost (C) to an altruist and benefit (B) to the recipient of an act are ranged as the change in the proportion of progeny produced, then the threshold for selection to favour a brother helping a sister ($C < B/2$) is lower than for a sister helping a brother ($C < B/4$). By similar reasoning, a female will be selfish to a brother at a higher cost (to recipient) to benefit (to actor) ratio than he will be to her; thus she is more likely to push him away from the food than vice versa. It is not known whether males feed less because they are altruistic or because females are selfish. However, female selfishness is suggested in that male

mortality is greater and in that males are more likely to have deformed antennae, legs or wings. Eleven of 308 females were dead compared with 25 of 292 males.

Conflicts are expected between a mother and her offspring, particularly her daughters, over the allocation of host resources. The distribution of adult sizes produced from a host that would give a mother her highest inclusive fitness is not necessarily the distribution that would give an individual brood member its highest inclusive fitness. Let a certain amount of food, if eaten by a male, increase his expected reproductive success by B_m and, if eaten by a female, increase her expected reproductive success by B_f . Sisters are selected to allow each other to feed in preference to brothers more often than their mother would be selected to allow them to do so. Applying the appropriate coefficients of relatedness, sisters favour a female feeding whenever $3/4B_f > 1/4B_m$, while a mother favours a daughter feeding only when $1/2B_f > 1/2B_m$. Thus a conflict between a mother and her daughters occurs when $3B_f > B_m > B_f$. There is no similar conflict between a mother and her sons: all are favoured if a female feeds when $B_f > B_m$ and a male feeds when $B_m > B_f$. Individuals of both sexes are selected to be more selfish than selection on the mother favours^{18,19}. When individuals benefit from eating the food themselves by B_s , a male is selected to eat in preference to his brother when $B_s > B_m/2$ and to his sister when $B_s > B_f/2$; a female is selected to eat in preference to her brother when $B_s > B_m/4$ and to her sister when $B_s > 3/4B_f$. A mother is selected to allow an individual to feed when $B_s > B_m$ and $B_s > B_f$. Because the mother is absent, it appears that the parasitoid hosts are divided up by the offspring.

The sexes were not distributed among broods at random but were clumped within broods. In general some broods contained more females than would be expected and others more males (Fig. 1). This skewing of brood sex ratios increases the average coefficient of relatedness within sibling groups and reduces contact between asymmetrically related brothers and sisters. As females of many Hymenoptera species accurately control the sex of their offspring by controlling fertilisation^{13,14,20}, female *P. stupidus* may have been selected to adjust brood sex ratios to reduce the number of situations in which there is a conflict with offspring over the distribution of resources. Daughters are selected to take a greater proportion of the resources than their mother is selected to give them^{4,17,21-23}. The observed distribution of brood sex ratios suggest that more resources went to males than would have, had broods been comprised of equal numbers of each sex.

I thank J. Brokaw, S. Guerrero and J. Wenzel for help with data collection, S. Bartz, W. Hamilton, P. Harvey, E. Leigh, R. Lewontin, C. Michener, J. Seger and J. Waage for comments and J. Seger for the analysis in Fig. 1. A predoctoral fellowship from the Smithsonian Tropical Research Institute, Panama, a National Science Foundation grant to R. Trivers, and the Anderson Fund, Harvard University, provided support.

Received 22 December 1978; accepted 2 November 1979.

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Chemical modification reduces the conductance of sodium channels in nerve

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Tetrodotoxin (TTX) and saxitoxin (STX) are extremely potent poisons that prevent nerve and muscle cells from producing action potentials by blocking sodium channels^{1,2}. If the channels are modified by reagents that act on carboxyl groups, however, both the binding of these toxins^{3,4} and their effect on the action potential⁵ are reduced. One such reagent, trimethyloxonium ion (TMO) converts channels into a form that is not blocked by TTX concentrations 10^5 times greater than its normal K_d (ref. 6). Most such chemical modifications of sodium channels also reduce the measured membrane sodium current, but it has not been known whether such reductions were due to a change in the number of channels, in permeability properties, or in gating properties. We now report that TMO-modified, TTX-resistant sodium channels have a smaller single-channel conductance (γ) with a more linear instantaneous current-voltage relationship than that of normal channels, and that the measured reduction in γ accounts for all of the decrease in sodium current after TMO treatment. This change in sodium channel permeability properties can be explained by the removal of a fixed negative charge near the outside of the channel.

Single nodes of Ranvier from the frog *Rana pipiens* were voltage-clamped by the method of Dodge and Frankenhaeuser⁷. Single-channel current amplitudes were estimated by ensemble fluctuation analysis⁸ from the mean current variance in sets of 100–500 current records from identical depolarisations. The analysis yielded estimates for the single-channel current i and the total number of channels N available for conduction. The single-channel chord conductance γ was calculated from values of i obtained at membrane potentials near 0 mV.

Brief treatment of nerve fibres with TMO ($(\text{CH}_3)_3\text{O}^+$), reduced the size of the sodium currents in Ringer solution, and the currents were reduced further when 200 nM TTX was added to block nearly all of the unmodified channels (Fig. 1). As discussed below, the reduction in current after TMO treatment was completely accounted for by the conversion of channels with a normal single-channel conductance ($\gamma = 7.1$ pS) to TTX-resistant channels having a reduced single-channel conductance ($\gamma = 2.4 \pm 0.3$ pS) (Fig. 2). Unfortunately, the fluctuation analysis technique does not allow us to distinguish between a homogeneous population of channels and a population having a spectrum of γ values, but for the sake of simplicity we consider both the normal and modified channel populations to be homogeneous.

Table 1 compares the values of γ , N and peak current I_{peak} obtained with 70 mV depolarisations from rest before and after TMO treatment. Estimates for the number of unmodified and modified channels open at the peak, N_{open} , are also given. Values of N_{open} for each component were obtained from the ratios of the peak current to the corresponding single-channel current value. The total number of channels open at the peak is seen to be the same (within 10%) before and after TMO treatment. This

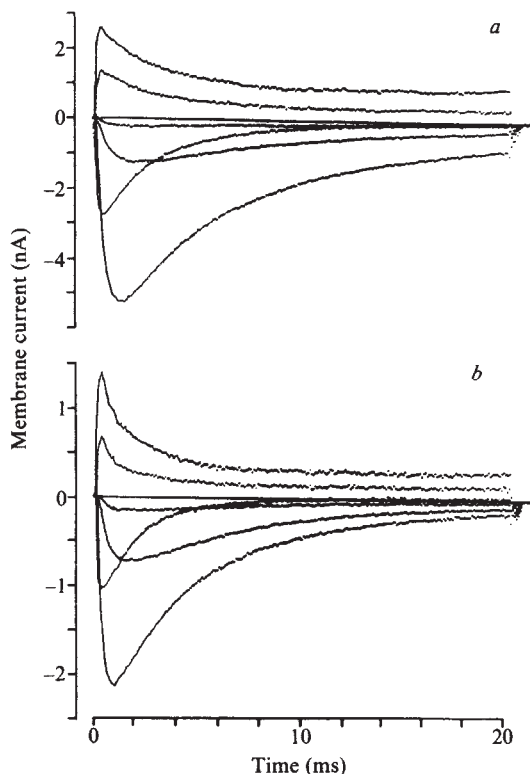


Fig. 1 Comparison of currents through *a* normal and *b* TMO-modified, TTX-resistant sodium channels in fibre 39 during 20 ms depolarisations to -45 , -35 , -15 , 25 , 85 and 125 mV from the holding potential of approximately -75 mV. Nerve fibres were treated with TMO as described in Table 1; the external solution in *b* contained 200 nM TTX. Fibres were voltage-clamped⁷ at 5°C in a Ringer solution containing 100 mM Na^+ as well as 20 mM tetraethylammonium ion (TEA) to block potassium current^{9,10}. TEA was also present in the end pools. (TMO treatment reduced potassium current by less than 20%, and did not change its susceptibility to block by TEA.) A 30 ms prepulse to -105 mV preceded each depolarisation in *b*. Leakage and capacitance currents were subtracted using scaled current records obtained with 40 mV hyperpolarising pulses. The currents were signal-averaged and plotted with different vertical scales.

indicates that the only effect of the TMO treatment appears to be conversion of normal to TTX-resistant channels; the total number of available channels remains constant. These comparisons of numbers of open channels rely on the observation, described below, that channel gating is not appreciably changed by TMO treatment. Similar values for the fraction of channels modified by TMO are obtained from comparisons of the total number of unblocked channels (N) estimated from the current fluctuations. These estimates of N are not based on many assumptions about channel gating but tend to be less reliable than γ values because of their increased sensitivity to the magnitude of background noise⁸.

To determine whether TMO treatment changed the gating of sodium channels, we observed the voltage dependence of activation of the TTX-resistant currents. This voltage dependence corresponded, within 4 mV, to that for currents in the fibres before modification. The shorter activation and inactivation time constants seen in the TTX-resistant currents of Fig. 1 almost certainly arose from a small (2°C) temperature rise during the experiment, because the change was not consistently seen, and the voltage dependence of the activation and inactivation time constants was also essentially unchanged.

Although the sodium current reversal potential was unchanged (within 4 mV) by TMO treatment, the TTX-resistant currents showed relatively larger outward currents at large depolarisations. We believe that this deviation is due to a change in the permeability properties of open channels. The instantaneous current-voltage (I - V) relationship, generally assumed

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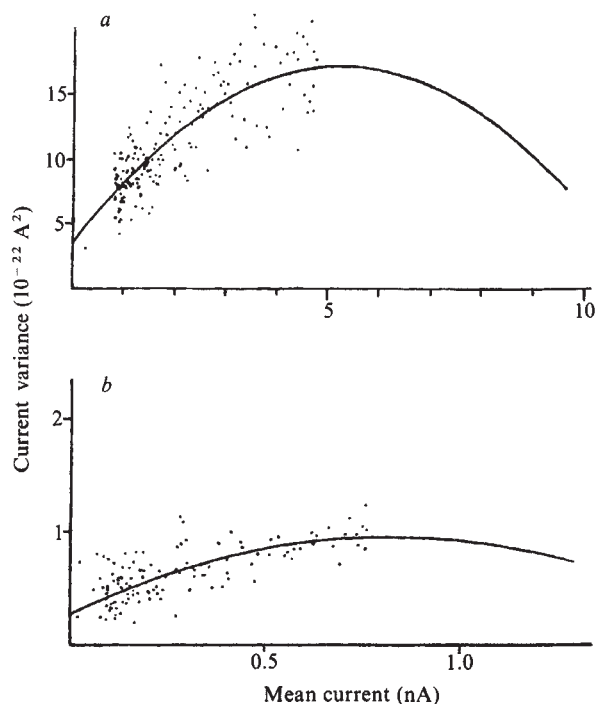


Fig. 2 Variance-mean current plots in normal and TTX-resistant sodium channels from fibre 43. Current variance and mean current values from depolarisations to -15 mV were obtained as described in Table 1. Smooth curves were obtained by fitting the points with the relation $\sigma_I^2 = \gamma(E - E_{Na})I - I^2/N$ after correction for background noise. Scales have been chosen so that a conductance of 5 pS corresponds to a slope of one in each part of the figure. The shallower slope in *b* reflects the smaller value of γ in TMO-modified channels. *a*, Before TMO treatment; the curve was drawn taking $\gamma = 7.0$ pS and $N = 20,800$. *b*, After TMO treatment and in the presence of 200 nM TTX; the curve was drawn taking $\gamma = 2.6$ pS and $N = 9,300$.

to reflect the ion permeation properties of the channel at a constant state of the gating system¹¹, is plotted together with estimates of i at various potentials in Fig. 3. The instantaneous I - V relationship is less curved for the TTX-resistant currents than for the control. The close correspondence between the voltage dependence of the instantaneous current and the single-channel current estimates, like that already seen in untreated fibres⁸, suggests that the modified channels have only one non-zero conductance level. Assuming this to be the case, we have scaled the instantaneous current curves in Fig. 3 using the single-channel current estimates, and identify the curves with the current-voltage relationship of single open channels.

We conclude that TMO modification of sodium channels changes the permeability properties of the channels, but leaves the gating properties essentially unchanged. The changes in single-channel conductance and rectification resemble those observed by Appel *et al.*¹² in hybrid channels formed from *O*-pyromellityl gramicidin A (OPG) and gramicidin A (G). They attributed the lower conductance and rectification of normal G-G channels, relative to hybrid OPG-G channels, to the absence of the electrostatic effects due to the negatively charged pyromellityl moiety. We expect that TMO removes a negative charge in the vicinity of the sodium channel by the esterification of a normally ionised carboxyl group. Normally, the negatively charged carboxyl group would increase the local cation concentrations near the outside of the sodium pore above that of the bulk solution. The modified channels, lacking this charge, would have a lower local concentration and would exhibit reduced conductance and rectification.

This view is supported by the observation that the effect of the hypothesised changes in the local potential and ion activities at the membrane surface may be partially simulated by suitable changes of the bulk solution potential and ionic activities. As

shown in Fig. 3, the substitution of 80% of the sodium by impermeant tetramethylammonium (TMA) ions produced single-channel currents in an untreated fibre similar in size and voltage-dependence to the toxin-resistant currents in standard Ringer solution. This simulation is not exact because the concentrations of the other ions were not also suitably changed, and because TMA, although it is better than other ions in this regard, may interfere with sodium ion permeation.

In another experiment, we investigated the relationship between the site at which TMO acts to reduce γ and the toxin binding site by pretreating fibre 105 with 200 nM TTX and then treating it with TMO in the presence of TTX. After a 10 min wash in toxin-free Ringer, peak sodium current returned to 40% of its original value and fluctuation analysis revealed no decrease in γ (Table 1). Following this treatment, application of 200 nM TTX reduced sodium current to undetectable levels. Apparently, TTX prevents TMO from reaching the site at which it acts to reduce γ . This experiment also shows that the decrease in γ is not due to nonspecific action of TMO, buffer solution, or the products of TMO hydrolysis (methanol and dimethyl ether). Furthermore, other experiments⁶ show that at least 80% of the sodium conductance returns on washing after treating a frog skeletal muscle fibre with TMO in the presence of STX, indicating that TMO treatment does not obstruct the movement of toxin between its receptor and the bulk solution. It seems likely that toxin resistance and reduced γ are both due to TMO action at the toxin binding site, although the possibility that TMO may act to reduce γ at a different site which is inaccessible in the presence of bound toxin cannot be ruled out.

Although in aqueous solution TMO reacts preferentially with carboxyl groups, it should be noted that the possibility that the effects we describe are due to reaction with other functional groups cannot be completely excluded. The specificity of TMO has been discussed more fully elsewhere⁶.

Reversible reductions in γ have previously been observed at low pH (ref. 8) and in the presence of Ni^{2+} (ref. 13). Small reductions in γ have also been observed in sodium channels with modified inactivation¹³. However, this is the first report of a chemical modification of sodium channels resulting in a large change in γ with essentially no change in channel gating. It will

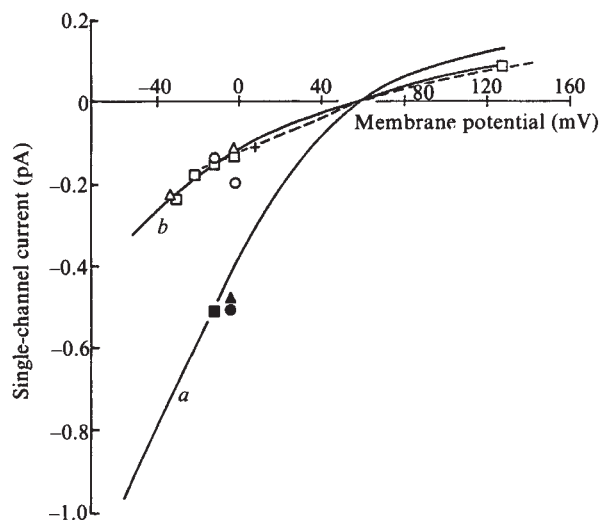


Fig. 3 Instantaneous current-voltage relations (curves) from fibre 43 and single-channel current estimates (symbols) from the same fibre ($\square$, $\blacksquare$) and from fibres 38 ($\circ$, $\bullet$) and 39 ($\triangle$, $\blacktriangle$). Filled symbols and curve *a*: normal currents, before modification with TMO; open symbols and curve *b*: TTX-resistant current; (+) and broken curve: normal currents before TMO modification from fibre 43 in one-fifth normal Na^+ (20 mM), shifted by 36 mV to superimpose the reversal potentials. The instantaneous current values, obtained at the test potential after a 1-m activating pulse to +45 mV, were scaled to fit the single-channel current values. The scaling factor is equivalent to the number of channels open at the end of the activating pulse.

Table 1 A comparison of sodium conductance before and after TMO treatment

Fibre	Treatment	N	γ (pS)	I_{peak} (nA)	Calculated N_{open}	
					Unmodified	Modified
38	Before TMO	22,000	7.3	7.0	13,900	—
	After TMO, no TTX	*	*	5.5	11,100	3,500
	After TMO, 100 nM TTX	7,000	3.1	0.6	—	3,500
39	Before TMO	41,000	7.2	12.0	25,600	—
	After TMO, no TTX	*	5.1*	6.5	10,300	13,100
	After TMO, 200 nM TTX	22,000	2.1	1.9	—	13,100
43	Before TMO	21,000	6.9	6.0	—	—
	After TMO, 200 nM TTX	10,000	2.3	0.8	—	—
105†	Before TMO + TTX	42,000	6.8	7.4	—	—
	After TMO + TTX, no TTX	17,000	7.5	2.9	—	—

Nerve fibres were treated with TMO by quickly dissolving the fluoborate salt (20 mM for fibre 38, 40 mM for fibres 39 and 43) in cold buffer solution (80 mM morpholinopropane sulphonic acid, MOPS) and applying this solution to the preparation. After 10 min the solution was washed out with Ringer solution. TMO reacts rapidly with water, so the concentration of reactive species that actually comes into contact with the membrane could be as little as one-half the values quoted. The tabulated values of N and γ were obtained by ensemble fluctuation analysis. A series of 100–500 sodium currents from repeated identical depolarisations was recorded, I , calculated by signal averaging, was subtracted, and the variance σ_i^2 was calculated for each point in groups of four digitised records. After a correction for background noise, the functional relationship between the variance and mean current was fitted by the quadratic function predicted by binomial statistics. For a homogeneous population of N independent channels having currents of 0 or i , the current variance is given by $\sigma_i^2 = iI - I^2/N$. The values of γ were calculated as the chord conductances, $\gamma = i/(E - E_{\text{Na}})$ where E , the membrane test potential, was chosen to be near 0 mV. E_{Na} is the reversal potential for current in sodium channels, which averaged 54 mV in these experiments. I_{peak} is the peak inward sodium current observed during a depolarisation to $E = -5$ mV, preceded by a 30 ms prepulse to -105 mV. N_{open} , the number of channels open at peak I_{Na} , was calculated for fibres 38 and 39 from measured values of I_{peak} and the corresponding γ values using the relation $N_{\text{open}} = I_{\text{peak}}/\gamma(E - E_{\text{Na}})$. The contribution of unmodified channels to the value of I_{peak} after TMO, in Ringer solution without TTX, was estimated by the difference between the I_{peak} values with and without TTX. The number of open, unmodified channels after TMO was therefore calculated as $N_{\text{open}} = (I_{\text{peak, no TTX}} - I_{\text{peak, TTX}})/\gamma(E - E_{\text{Na}})$, where the γ value from the untreated fibre was used.

* Only apparent values of N and γ can be measured after TMO treatment in the absence of TTX, since two populations of channels are present. The apparent γ value is an average of the γ values of each population, weighted by the current due to each population; the interpretation of apparent N values is less simple and these values have been omitted from the table.

† Fibre 105 was pretreated with 200 nM TTX and then treated for 10 min with 40 mM TMO dissolved in buffer solution containing 200 nM TTX. The values 'after TMO + TTX' were measured following a 10 min wash in toxin-free Ringer.

be interesting to see if naturally occurring toxin-resistant channels have properties similar to those of TMO-modified channels.

We thank Dr Charles F. Stevens for his encouragement and for laboratory facilities, Dr Bertil Hille for helpful advice, and Dr S. Y. Chiu for dissecting some of the fibres used in this study. This work was supported by grants NS 12962, NS 08174 and NS 14128 from the N.I.H.; B.C.S. was supported by a National Research Service Award from the NIH (GM 07270).

Received 16 April; accepted 8 November 1979

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Met-enkephalin circulates in human plasma

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The physiological roles of Met-enkephalin and Leu-enkephalin¹ are still unknown. They may act as neurotransmitters in the central and peripheral nervous systems². Met-enkephalin has been detected in several species in a variety of tissues including brain, spinal cord and gut using bioassays, opiate receptor assays and radioimmunoassays (RIA)^{3–8}. It has also been detected in human gut immunocytochemically and in human brain and cerebrospinal fluid by opiate receptor assay and RIA^{9–11}. However, all reported assays show some degree of cross-reaction with Leu-enkephalin and unequivocal differentiation between the two enkephalins and the larger endorphins has not always been possible. Thus the existence of Met-enkephalin in human tissues and fluids remains in doubt. Using a highly specific RIA, we have now obtained evidence that Met-enkephalin-like material circulates in the plasma of normal subjects and may be secreted by the adrenal gland. Chromatographically the material exists in plasma mainly as the intact pentapeptide and not as the biologically inactive degradation product Gly-Gly-Phe-Met as would be expected from metabolic studies^{12,13}.

Although bioassays and opiate receptor assays have the advantage of measuring biological and related activities they are unable to discriminate fully between related opiate peptides. To date all reported RIAs for Met-enkephalin cross-react with Leu-enkephalin and the larger endorphins. Thus to establish the presence or absence of Met-enkephalin in plasma we needed (1) to develop a sensitive RIA which did not cross-react with Leu-enkephalin and β -endorphin (the latter is known to be present in human CSF¹⁴ and plasma of normal subjects¹⁵); (2) to develop a system for extracting Met-enkephalin from plasma in order to concentrate it and to remove interference in the RIA by proteins and to minimise proteolysis; (3) to demonstrate any measured Met-enkephalin immunoreactivity is not an artefact of sample collection, extraction and the RIA system and (4) to demonstrate that any measured immunoreactivity extracted from plasma would co-elute on chromatography with standard Met-enkephalin. These aims have been achieved by the novel approach of developing a RIA to the methionine sulfoxide analogue of Met-enkephalin and oxidation of all samples by hydrogen peroxide before assay and after extraction by octadecasilyl-silica (ODS-silica). The RIA and extraction methodology will be described in detail elsewhere¹⁶.

We have observed that the C-terminal methionine residue of Met-enkephalin was unstable and underwent variable spontaneous oxidation to methionine sulfoxide (1–10%) during storage, extraction and assay procedures. No reported RIAs would detect this and yet, because of the oxidative conditions of iodination ¹²⁵I-labelled Met-enkephalin is likely to have a C-terminal methionine sulfoxide. We decided to raise high-titre antibodies to the methionine sulfoxide analogue of enkephalin (Met-O-enkephalin) in rabbits using as the antigen Met-O-enkephalin coupled via the N-terminus to bovine thyroglobulin by the glutaraldehyde method. This antibody does not cross-react with 1,000 molar excess of Leu-enkephalin, or with purified human β -lipotrophin (β -LPH) or β -endorphin (Fig. 1) before or after their oxidation by hydrogen peroxide. Purified human LPH 61–79 cross-reacts minimally (0.02%) after oxidation. There is minimal cross-reaction with synthetic α -endorphin (0.025%) and β -endorphin (0.25%) after oxidation. We

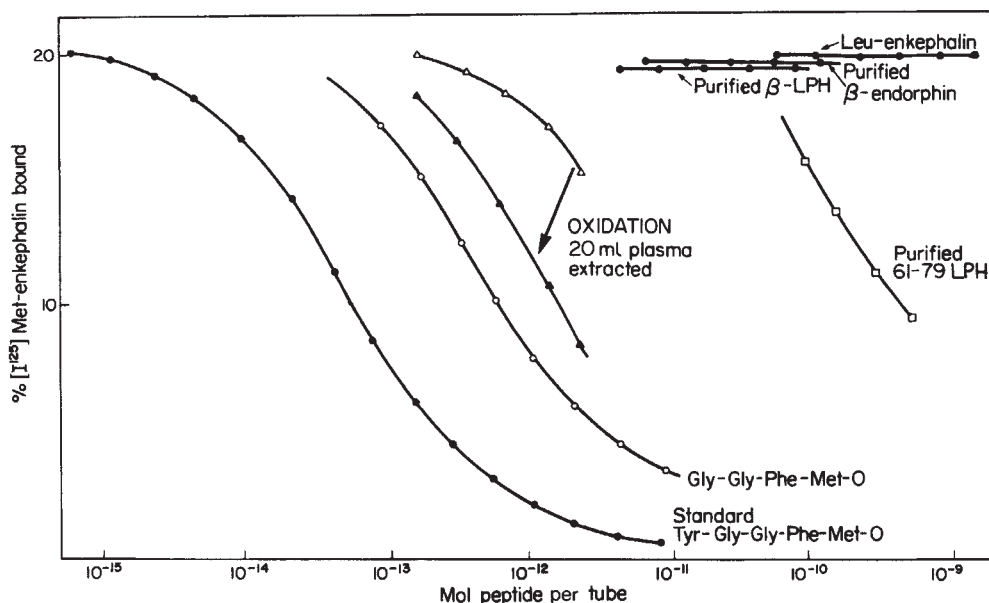


Fig. 1 Standard curve for Met-O-enkephalin and cross-reactivity studies. 50 μ l of antiserum (1 in 9,000 final dilution for antibody E2) was added to 200 μ l buffer containing doubling dilutions of standard Met-O-enkephalin and incubated for 24 h at 4 °C. Mono-iodinated Met-enkephalin (10 pg in 50 μ l diluent buffer) was added with further incubation for 24 h before separation of bound from free [125 I] Met-enkephalin by double antibody precipitation. The cross-reactivities of Leu-enkephalin, purified human β -LPH, LPH 61-79 and β -endorphin and Gly-Gly-Phe-Met are shown after oxidation by 100 nmol hydrogen peroxide. A sample of 20 ml of plasma from a normal subject was extracted in duplicate on ODS-silica (as described in

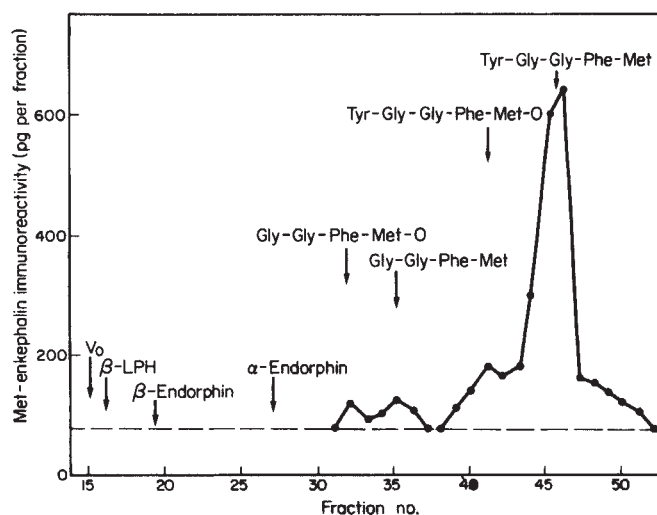
legend to Fig. 2) and the 80% methanol/1% formic eluates treated with ($\blacktriangle$) or without ($\triangle$) 100 nmol hydrogen peroxide before drying down by air, reconstitution and assay for Met-enkephalin.

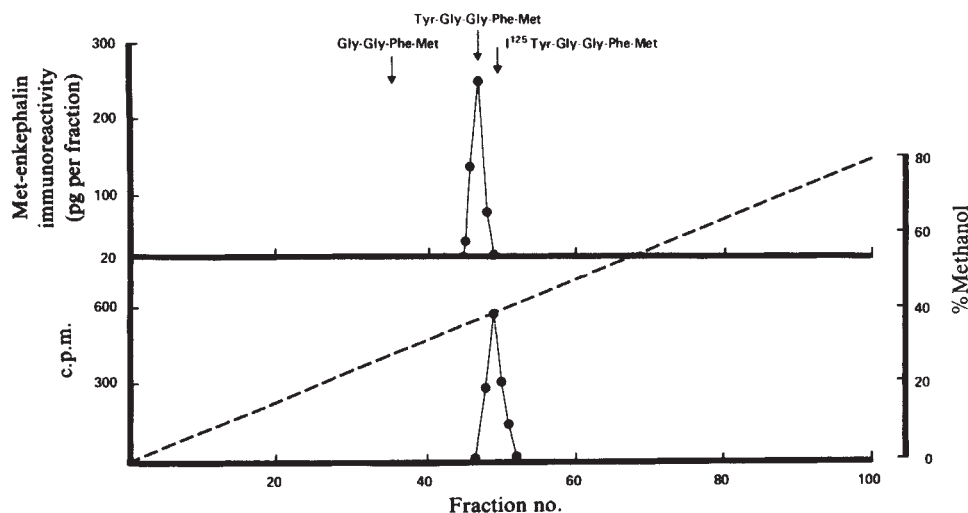
have found that 60% and 90% respectively of their cross-reaction is due to the contamination of these synthetic preparations by Met-enkephalin. Using this antibody and mono-iodinated Met-enkephalin tracer highly purified on ODS-silica (10- μ m particles) the RIA has a sensitivity of 0.01 pmol ml $^{-1}$. Our extraction system uses coarse ODS-silica (35-70 μ m) in 1% formic acid. It allows concentration of 1-25 ml of plasma, is able to exclude the nonspecific depression of binding of tracer by plasma proteins and minimises proteolytic activity by virtue of being carried out at pH 1.5. This system also maximises the recovery of Met-enkephalin since the larger peptides β -LPH and β -endorphin elute poorly from coarse ODS-silica with 80% methanol/1% formic acid. When Met-O-enkephalin is added to 1 ml plasma and extracted there is 80-90% recovery. The poorer recovery of Met-enkephalin (20-30%) after extraction is related to losses during the oxida-

tion by hydrogen peroxide. Our extraction and assay system has the inbuilt control of increased immunoreactivity diluting in parallel after treatment with hydrogen peroxide if the material is Met-enkephalin.

With this extraction and assay system and suitable collection of plasma samples to minimise degradation, 4 ml plasma samples from 20 normal subjects taken at 9 a.m. yielded Met-enkephalin immunoreactivity of 14-140 pg ml $^{-1}$. Repeated assay of samples from the same plasma pool gave an intra-assay variation of 10.2% ($n = 8$) at a mean value of 22 pg ml $^{-1}$. When 20 ml of plasma was extracted and treated with or without H $_2$ O $_2$ an increase in immunoreactivity with H $_2$ O $_2$ was seen (Fig. 1) suggesting this material is Met-enkephalin. The assay does have a slight cross-reaction (0.5%) with the tetrapeptide Gly-Gly-Phe-Met and 10% with Gly-Gly-Phe-Met-O. There was no cross-reaction however with smaller fragments of Met-enke-

Fig. 2 A 100 $\times$ 1 cm BioGel P4 (200-400 mesh) column was equilibrated with 1% formic acid and pumped at room temperature at 3 ml h $^{-1}$. The column was presaturated with human serum albumin 3 mg (HSA, Blood Products Laboratory, Elstree) and calibrated initially for standard synthetic Met-enkephalin (Bachem) and then synthetic Gly-Gly-Phe-Met (Reckitt and Colman). The amino acid composition of both peptides was checked by amino acid analysis after acid hydrolysis. Fractions of 2 ml were collected and assayed for Met-O-enkephalin after oxidation by 100 nM H $_2$ O $_2$, drying down with air and reconstitution with 0.05 M sodium phosphate pH 7.4 containing 0.25% HSA and 0.1% 2-mercaptoethanol and phenol red as indicator. V_0 indicates the void volume as shown by the HSA marker. Purified β -LPH, β -endorphin and synthetic α -endorphin were loaded and elution pattern determined by RIA of N- and C-terminal β -LPH 14,21 and α -endorphin. Samples of 160 ml of whole blood were collected from normal subjects at 9 a.m. into 10 ml lithium heparin tubes containing 10,000 KIU (Kallikrein inactivator unit) aprotinin (Trasylol) and centrifuged immediately at 4 °C. Pooled plasma (80 ml) was acidified to pH 1.5 by the addition of 150 μ l glycine-HCl (1.6% glycine in 1M HCl) per ml of plasma and formic acid to final concentration of 1%. Plasma was extracted and concentrated in 8-ml batches on 1.5 $\times$ 0.5-cm columns containing 35-70 μ m ODS-silica 16 . Acidified plasma was loaded in 800 μ l aliquots followed by 800 μ l of a solution of 0.9% NaCl and 1% formic acid containing 1.5 ml glycine-HCl per 100 ml. After loading 8 ml of acidified plasma a final wash with 800 μ l 1% formic acid was followed by 1.6 ml of 80% methanol/1% formic acid which eluted the adsorbed peptides into a round-bottomed plastic tube containing 100 μ g polypeptide (Sigma Polypep P5115) and 10 mg mannitol. Each 1,600 μ l eluate was evaporated to dryness by oxygen-free nitrogen at 50 °C and each reconstituted in 200 μ l 1% formic acid. After centrifugation at 2,000g the 2 ml of combined supernatant was loaded on BioGel P4 column after addition of 3 mg HSA. The column was eluted with 1% formic acid and pumped at 3 ml h $^{-1}$ at room temperature and 2 ml fractions collected. H $_2$ O $_2$ 100 nmol was added to each fraction and left for 12 h before evaporation by compressed air in a sandbath at 50 °C and assay in duplicate for Met-O-enkephalin after reconstitution in assay buffer. If required pH was corrected to 7.4 by dropwise addition of 1.1M NaHCO $_3$.





indicate the elution positions of synthetic Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Met and monoradioiodinated Tyr-Gly-Gly-Phe-Met appearing at fractions 35, 47 and 49 respectively in this system.

phalin¹⁶. Thus we could be detecting the inactive Gly-Gly-Phe-Met which, from degradation studies, might be the expected main circulating form of Met-enkephalin¹². To examine this possibility we subjected plasma concentrated on ODS-silica to chromatography on BioGel P4. We initially calibrated the column for synthetic Met-enkephalin, Gly-Gly-Phe-Met, β -LPH, β -endorphin and α -endorphin as shown in Fig. 2. Met-enkephalin elutes in fraction 45 with a smaller peak at fraction 42 corresponding to Met-O-enkephalin generated by spontaneous oxidation. The elution of Met-enkephalin is retarded on BioGel P4 because of its hydrophobicity. This would explain the earlier elution position of Met-O-enkephalin which is less hydrophobic. Gly-Gly-Phe-Met similarly elutes into two peaks of oxidised and native peptide but well separated from the peaks of Met-enkephalin.

Figure 2 shows the chromatography of 80 ml of ODS-extracted plasma on BioGel P4. The elution profile shows the main peak of immunoreactivity at fraction 45 co-eluting with the known position of Met-enkephalin and a smaller peak at fraction 35 corresponding to the position of Gly-Gly-Phe-Met. There are also small peaks corresponding to Met-O-enkephalin and Gly-Gly-Phe-Met-O; these might be expected from spontaneous oxidation during extraction and chromatography. Further evidence for the identity of the main peak from the BioGel P4 column was obtained from reverse phase HPLC (Fig. 3).

These data suggest that the bulk of immunoreactivity in plasma after extraction in conditions which inhibit proteolytic degradation is Met-enkephalin (85%), a small amount (15%) may be due to the tetrapeptide Gly-Gly-Phe-Met which has lower cross-reactivity. Since we can demonstrate that the recovery after ODS extraction of Gly-Gly-Phe-Met and Met-enkephalin added to acidified plasma is equal we cannot have preferentially extracted either peptide before BioGel P4 chromatography.

When human plasma containing exogenous or endogenous β -LPH or β -endorphin was incubated for several hours at room temperature¹⁶ we failed to detect an increase in Met-enkephalin immunoreactivity suggesting that the Met-enkephalin we detect in plasma is unlikely to be an artefact of our extraction system formed by degradation of plasma β -LPH or β -endorphin.

Dexamethasone treatment (2 mg at midnight) of seven normal subjects suppressed plasma immunoreactive ACTH and β -LPH to undetectable levels but did not alter the circulating Met-enkephalin levels significantly ($P > 0.2$). Samples collected from two normal subjects at 9 a.m. and midnight did not show a nyctohemeral rhythm in Met-enkephalin secretion as is observed for ACTH and β -LPH¹⁷. These data suggest that the Met-enkephalin-like immunoreactivity in plasma is independent of circulating ACTH, β -LPH and β -endorphin, which have

Fig. 3 High-pressure liquid chromatography (Pye Unicam LCXP) of the main plasma Met-enkephalin peak from BioGel P4 chromatography. The column (50 mm $\times$ 4 mm, Spherisorb ODS 10 μ m) was developed with a linear gradient (---) from 1% trifluoroacetic acid to 80% methanol/1% trifluoroacetic acid at 2 ml min⁻¹ collecting 0.2 min fractions. All fractions were assayed for Met-enkephalin as described in the legend to Fig. 2. The limit of detection was 20 pg per fraction. Immunoreactive enkephalin eluted in the expected position of synthetic Met-enkephalin (upper chromatogram) two fractions earlier than 2 pg of monoradioiodinated Met-enkephalin¹⁶ (lower chromatogram) added as internal marker. The arrows

been shown to be secreted in parallel from the pituitary gland in response to different stimuli¹⁸. To determine the source of circulating Met-enkephalin we measured samples collected at selective venous catheterisation of a patient undergoing investigation for hirsuties. Although the level of Met-enkephalin in venous blood taken from the left internal jugular (55 pg ml⁻¹) was the same as the peripheral level (53 pg ml⁻¹), simultaneous peripheral and left adrenal vein samples showed twice the concentration in the adrenal effluent (116 pg ml⁻¹). These results suggest that Met-enkephalin is secreted into the circulation from the adrenal glands. Immunoreactive Met-enkephalin has been detected in whole rat adrenal by RIA¹⁹. Furthermore immunocytochemical studies of the adrenal show the Met-enkephalin located mainly in the adrenal medulla²⁰. Fresh human adrenal tissue was carefully dissected into medulla and cortex and each homogenised in boiling 0.1 M HCl. After the addition of formic acid to the supernatant to final concentration of 1%, Met-enkephalin was measured after coarse ODS extraction, using the method as described for plasma. The majority of the immunoreactive Met-enkephalin was located in the medulla (0.8 μ g per g) with small but detectable amounts in the cortex (0.02 μ g per g).

We conclude that Met-enkephalin circulates as the intact pentapeptide in human plasma and is possibly secreted by the adrenal medulla. Plasma levels of Met-enkephalin are unrelated to the levels of endogenous ACTH, β -LPH and β -endorphin which adds further support to the argument that Met-enkephalin belongs to a separate neuropeptide system with its own precursor.

Received 12 June; accepted 26 November 1979.

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GABA and picrotoxinin receptors in clonal nerve cells

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γ -Aminobutyric acid (GABA) is a neurotransmitter which seems to mediate inhibitory synaptic transmission in many regions of the central nervous system (CNS) of vertebrates and invertebrates¹. GABA has been shown to act by increasing membrane chloride permeability in invertebrate muscle^{2,3}, and a similar mechanism has been suggested for the vertebrate CNS⁴. The response to GABA is inhibited noncompetitively by the convulsant picrotoxinin¹⁻⁵. Binding sites for radioactive GABA⁶⁻⁸ and picrotoxinin⁹⁻¹¹ (the active component of picrotoxin) have been described in homogenates of invertebrate muscle and mammalian CNS; these binding sites show many properties expected of postsynaptic membrane macromolecules involved in the receptor-ionophore function of GABA. Nevertheless, unambiguous correlation between biochemical assays and *in vivo* function is difficult to obtain, especially in heterogeneous tissue such as mammalian CNS. One approach to this problem involves cloned cell lines of neuronal origin which retain in culture the differentiated function to be studied (see, for example, ref. 12). Certain cell lines cloned from rat¹³ and mouse¹⁴ nervous system possess neuronal properties such as electrical excitability, neurotransmitter synthetic enzymes¹², neurotransmitter uptake^{15,16}, binding of defined ligands to their appropriate neurotransmitter receptors^{12,17,18}, and receptor-coupled responses to neurotransmitters^{17,19,20}. Several cell lines of apparent glial origin have also been described^{13,21,22}. To determine whether binding sites for GABA and picrotoxinin are localised to nerve cells, a variety of clonal cell lines were assayed for their ability to bind those ligands. We report here the presence of GABA and picrotoxinin binding sites with receptor-like properties in several clonal nerve cell lines obtained from the rat CNS, and the absence of such binding sites in many other cell lines of non-neuronal origin.

Table 1 shows that GABA receptor-like binding and α -dihydropicrotoxinin (DHP) binding were present in membranes from all five rat CNS nerve lines examined, whereas no binding was seen with the non-neuronal lines. GABA binding was measured as that fraction of radioactive GABA pelleted with the membranes that was displaceable by 10^{-5} M muscimol, in sodium-free buffer, using membranes thoroughly washed with buffer and repeatedly frozen and thawed. Extensive washing is required to remove endogenous inhibitors of GABA binding that were seen in the extracts of these cells (data not shown), as previously observed in brain⁸. Binding of GABA in such conditions has been well demonstrated to reflect receptor-like specificity^{6-8,23}.

Binding of ³H-GABA and ³H-DHP to one of the nerve cell lines, B35, was studied in detail for comparison with the well characterised binding of these ligands to rat brain homogenates. Figure 1 shows a Scatchard plot of sodium-independent GABA-displaceable binding against GABA concentration for B35 membranes. An apparent single class of affinity ($K_D = 22$ nM) was observed, with a total capacity of sites (maximal binding at saturating ligand concentration) of 250 pmol per g protein. This quantity of sites is roughly equal to the total number of sites in some of the less dense regions in CNS²³⁻²⁵. Assuming that levels of binding activity can be compared in one set of constant assay conditions, the level in the other four lines tested was slightly lower (Table 1). The absolute amount of specific GABA binding did, however, vary between different preparations of the same

cell line. This is probably due to slight differences in growth state and is under investigation. The GABA binding affinity to B35 membranes was equal to that of one of the two classes of sites in mammalian brain which have K_D values of 20 and 180 nM (refs 8, 25). No low-affinity GABA binding corresponding to the brain site having a K_D of 180 nM could be detected in B35 cell membranes in three experiments. Considering only the high-affinity GABA binding sites, the number of sites in B35 cells was about the same as that for this class of sites in high-density brain regions²⁵.

Figure 1 (inset) shows that this high-affinity sodium-independent GABA binding to B35 cells was displaced by GABA analogues with the specificity expected of receptor sites. The receptor-specific drugs muscimol ($IC_{50} = 50$ nM in B35, 25 ± 15 nM in brain)⁷ and 3-aminopropane sulphonate ($IC_{50} = 500$ nM in B35, 85 ± 15 nM in brain)⁷ were good inhibitors, with concentrations $>10^{-5}$ M displacing the same amount of ³H-GABA as did 10^{-4} M nonradioactive GABA. However, the transport-specific analogue 2,4-diaminobutyrate was not active ($IC_{50} > 10^{-4}$ M in both brain and cell line B35). Bicuculline was a modest inhibitor of GABA binding in B35 and picrotoxinin had no effect up to 10^{-4} M.

Table 1 Specific GABA and DHP binding to membranes from cultured cell lines

Cell line	Cell type	DHP bound GABA bound	
		Ref.	(fmol per mg protein)
B35	CNS nerve	13	850 $\pm$ 100 130 $\pm$ 20
B50	CNS nerve	13	513 $\pm$ 90 77 $\pm$ 10
B65	CNS nerve	13	378 $\pm$ 80 25 $\pm$ 3
B103	CNS nerve	13	394 $\pm$ 80 48 $\pm$ 4
B104	CNS nerve	13	252 $\pm$ 50 32 $\pm$ 4
B9	CNS glial	13	NS NS
B12	CNS glial	13	NS NS
B15	CNS glial	13	NS NS
B82	CNS glial	13	NS NS
PC12	Phaeochromocytoma		
	exponentially dividing	27	NS NS
PC12	Stationary phase	27	NS NS
L6	Skeletal myoblast	28	NS NS
S194/5	Plasmacytoma	29	NS NS

The clonal cell lines were grown as described in the references indicated. With the exception of PC12, which was collected at both the exponential (1×10^6 cells per 100-mm diameter dish) and stationary phase of growth, all cells were collected from early stationary phase cultures, 2 days after cessation of net cell division, at a density of $4-6 \times 10^6$ cells per 100-mm diameter dish. The cells were greater than 95% viable as measured by the uptake of fluorescein dibutyrate. During preparation, the cells were washed twice in phosphate-buffered saline (150 mM NaCl, 10 mM Na phosphate, pH 7.3, 2 mM CaCl₂, 0.5 mM MgCl₂), frozen as a pellet (0.2-1 g) on dry ice and stored at -85°C . These were thawed in 5 ml distilled H₂O and sonicated twice for 30 s (MSE sonifier, maximum setting) at $0-4^\circ\text{C}$ separated by a 30 s cooling break. The suspension was then homogenised by hand at 0°C with five passes of a glass-glass Dounce homogeniser, diluted to 9 ml and centrifuged for 30 min at 200,000g [Beckman rotor 65 (49,000 r.p.m.)]. For DHP binding studies, the pellet was rehomogenised at $0-4^\circ\text{C}$ with a Potter-Elvehjem homogenising tube and motor-driven pestle, five passes at 600 r.p.m., in 0.2 M NaCl, 10 mM Na phosphate, pH 7.0, centrifuged as above and rehomogenised as before in the same buffer for assay¹⁰. For GABA binding assays⁸, the first pellet obtained after homogenisation was rehomogenised in 10 ml of 50 mM Tris buffered to pH 7.1 at 4°C with citric acid (sodium-free), and frozen at least 15 h. On the day of assay, this homogenate was thawed, pelleted as before, rehomogenised in the same buffer and repelleted, then rehomogenised in the same buffer for assay. The washing procedure is suitable for the removal of endogenous inhibitors of GABA binding in brain membranes⁸; further washing of the membranes obtained from the cells did not alter GABA binding. The protein concentration was adjusted to 0.5 mg in 1 ml assay volume. Binding of both ligands was determined by centrifugation at 48,000g (20,000 r.p.m., Sorval rotor SM24) following incubation in triplicate at $0-4^\circ\text{C}$ for 15 min, at which time equilibrium was obtained^{8,9}. ³H-GABA (Amersham, 54 Ci mmol⁻¹) was included at 9.3 nM, with background (nondisplaceable radioactivity pelleted) estimated with 10^{-5} M muscimol. The nondisplaceable background was approximately 4,000 c.p.m. in each case, and the specific binding varied over 4,000-12,000 c.p.m. ³H-DHP (Amersham, 26 Ci mmol⁻¹) was 63 nM, with background estimated by 10^{-4} M nonradioactive DHP. Background was generally 20,000 c.p.m., and specific binding varied over 3,000-10,000 c.p.m. After pelleting, the tubes were superficially rinsed twice, not disturbing the pellets, with 5 ml assay buffer, and the pellets were dissolved overnight at 22°C in 0.1 ml Soluene-350 (Packard). Radioactivity was measured in a Beckman LS-100C scintillation counter with an efficiency of 40%, using a 0.5% diphenyloxazole PPO in toluene. Each value is the mean of two experiments in triplicate, $\pm$ standard deviation. NS, not significant at 90% confidence level (<100 fmol DHP bound per mg protein or <5 fmol GABA bound per mg protein at the concentrations of ligand used).

Table 2 Drug inhibition of DHP binding to B35 clonal nerve cells

Displacing agent (M)	Per cent inhibition of specific binding				
	DHP	Picro-toxinin	DMBB	Mephobarbital	IPTBO
10^{-7}	5 ± 5	20 ± 5	—	—	—
5×10^{-7}	42 ± 10	—	—	—	—
10^{-6}	—	—	—	—	—
2×10^{-6}	84 ± 20	—	—	—	—
10^{-5}	93 ± 20	87 ± 20	106 ± 20	—	—
10^{-4}	100 ± 20	—	—	76 ± 20	66 ± 20

Membranes were prepared from cells grown as described in Table 1, and assayed for ^3H -DHP binding¹⁰ in the presence of various concentrations of the drugs indicated. DHP, nonradioactive α -dihydropicrotoxinin; DMBB, 1,3-dimethylbutylbarbiturate; IPTBO, isopropylbicyclopophosphate. ^3H -DHP was 63 nM, 26 ± 5 Ci mmol⁻¹; protein 0.5 mg in 1 ml assay volume. The controls gave roughly 8,000 c.p.m. bound and a background of 20,000 c.p.m. Each value is the mean of two experiments done in triplicates.

These specificity properties are similar but not identical to the properties described for rat brain⁷. The small differences may reflect the presence of fewer classes of binding site populations in the B35 cells than in brain, and/or the receptor properties in the cells may be different because of a location in membranes with incompletely differentiated synaptic characteristics. Slight modifications unique to the cell line or its growth conditions are also possible.

The DHP binding to B35 cells was also further characterised (Table 2). Properties similar to those found for the binding sites in brain were observed, with 50% displacement by about 10^{-6} M nonradioactive DHP (brain $K_D = 1.2 \times 10^{-6}$ M)¹⁰, and by 10^{-6} M picrotoxinin (brain $\text{IC}_{50} = 0.4 \times 10^{-6}$ M)¹⁰, and 100% displacement by 10^{-5} M 1,3-dimethylbutylbarbiturate (brain $\text{IC}_{50} = 5 \times 10^{-8}$ M)¹¹, 76% displacement by 10^{-4} M mephobarbital (brain $\text{IC}_{50} = 5 \times 10^{-6}$ M)¹¹ and 66% displacement by 10^{-4} M isopropylbicyclopophosphate (brain $\text{IC}_{50} = 8 \times 10^{-6}$ M)¹⁰.

Thus, both GABA and DHP binding sites in B35 membranes had properties similar to those characterised in brain. The specific GABA receptor and DHP binding sites were observed only in cell lines of CNS origin which have neuronal properties, and were absent from several other types of cell. The above results open the way for further studies of this neurotransmitter using the clonal cell cultures. This system should facilitate the study of several questions regarding GABA function, such as

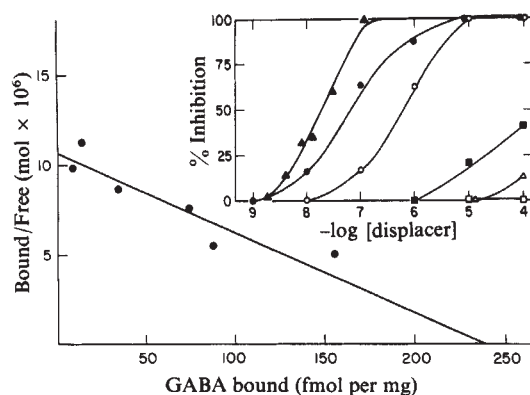


Fig. 1 Scatchard plot of GABA binding to B35 clonal nerve cells. Membranes were prepared and assayed⁸ as described in Table 1 legend. Protein was 0.7 mg in 1 ml assay volume, ^3H -GABA (54 Ci mmol⁻¹) was varied over 0.1–10 nM, and nonradioactive GABA over 10 nM–2 μM , with 10^{-4} M nonradioactive GABA used to estimate nondisplaceable background. Each point is the mean of triplicates and the experiment is typical of three. The ratio of specific binding to background varied from 3.0 (low concentrations) to 0.4 (high concentrations), with standard deviations of $\leq 4\%$ in the raw data and $\leq 10\%$ in the specific binding. Inset: receptor specificity of GABA binding to B35 neuroblastoma cells. ^3H -GABA was 9.3 nM. Control binding was about 6,000 c.p.m. with background of 4,000 c.p.m. Binding was measured in the presence of various concentrations of displacing agent. Each point is the mean of triplicates and typical of two experiments. ●, Muscimol; ○, 3-aminopropane sulphonate; △, 2,4-diaminobutyrate; ■, bicuculline; □, picrotoxinin; ▲, GABA.

the regulation of chloride permeability, the heterogeneity in binding sites, the function of endogenous inhibitors⁸ and the interactions with barbiturates¹¹ and benzodiazepines²⁶.

This work was supported by grants from the NIH, NSF and Muscular Dystrophy Association of America. IPTBO was from Dr J. Casida, muscimol from Dr P. Krogsgaard-Larsen, 3-aminopropane sulphonate from Dr G. A. R. Johnston, DMBB from Lilly and mephobarbital from Sterling-Winthrop.

Received 26 April; accepted 15 November 1979.

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Quantitative microlocation of lithium in the brain by a (n, α) nuclear reaction

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Although lithium has long been used in psychiatric practice^{1–4}, its exact mechanism of action remains largely unknown. The reason is mainly methodological. Lithium has no radioisotope, and it is too light to be detectable by electron-probe microanalysis. To study the distribution of lithium in the brain, most authors⁵ have proceeded by dissecting the brain into small fragments and determining the mean Li content of each fragment by photometric methods. However, the resolving power by this method has remained poor. Our group⁶ and others⁷ have recently described the possibility of using the stable isotopes of lithium as tracers for estimating unidirectional fluxes. To study Li-location, there is the theoretical possibility of using the specific nuclear reaction $^6\text{Li}(n, \alpha)^3\text{H}$. The method cannot be used as a conventional radioactivation because the induced ^3H has negligible radioactivity compared with the many other radioisotopes also induced in a biological sample bombarded by neutrons. The detection then consists of laying sections of the ^6Li -containing samples in contact with a convenient detector, and irradiating the whole arrangement with neutrons; the distribution of lithium in the sample is revealed by the tracks of the α and ^3H particles in the detector. Some authors have already attempted to use such methods for various biological applications^{8–15}. By taking advantage of different independent technical advances^{16–22}, we can now give a precise quantitative picture of the distribution of lithium in the brain of a lithium-treated mouse.

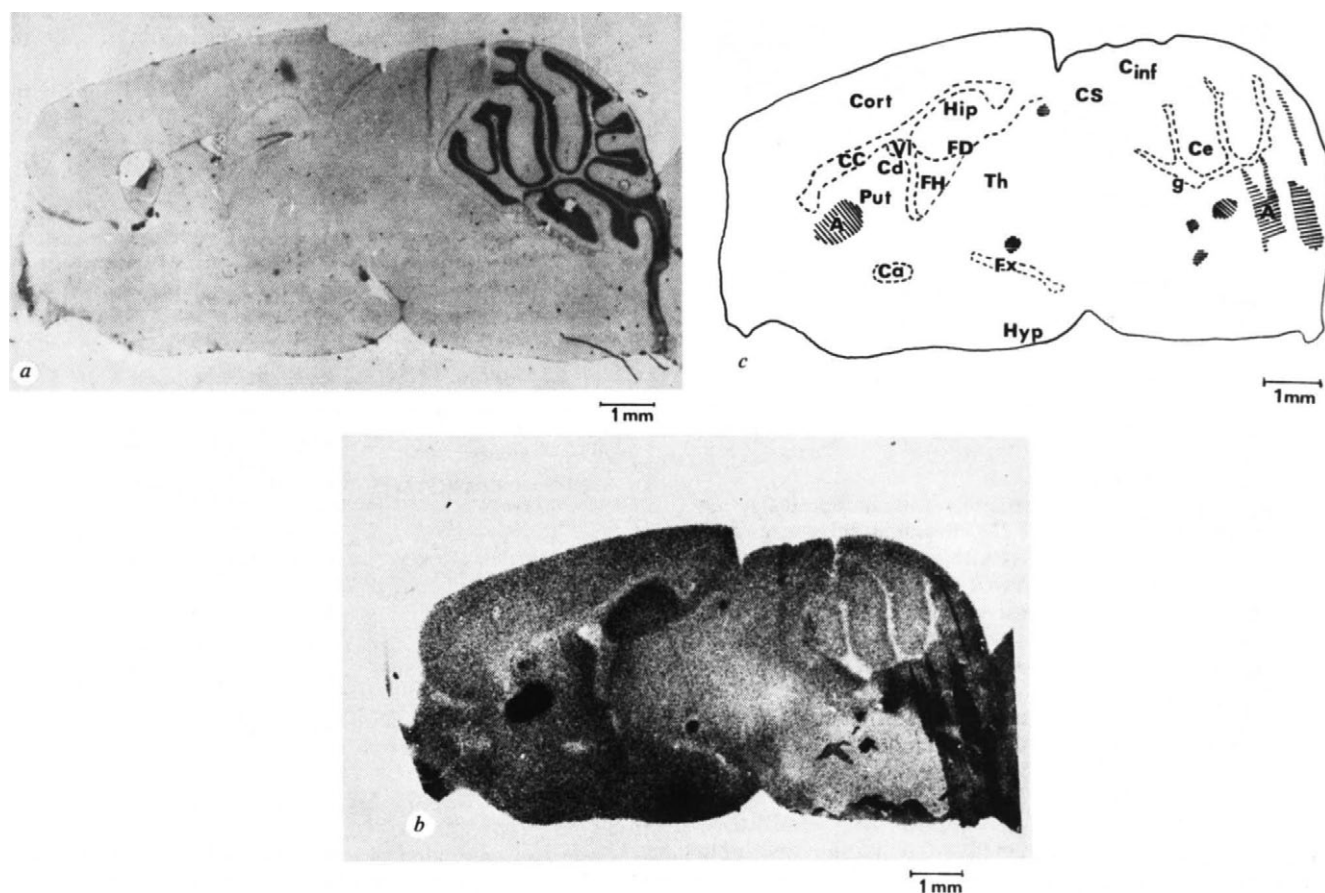


Fig. 1 Lithium distribution in a mouse brain. *a*, Histological preparation stained by Feulgen method. *b*, Photograph of the corresponding detector (each small black spot which forms the shading seen corresponds to a nuclear reaction on lithium). *c*, Schematic representation of the brain microanatomy at the level of the section. The mice had been treated with a daily dose of 12 mequiv. per kg lithium for 3 days, with the last dose 6 h before the experiment. Lithium was given as LiCl in the form of ^6Li -enriched isotope 96%. The mice were decapitated, and each brain was immediately removed and immersed in liquid isopentane cooled by liquid nitrogen. Sagittal sections (10 μm thick) of the frozen brains were made with a Jung microtome placed in a WKF chamber cooled to -35°C . The sections were deposited on quartz supports, lyophilised, tightly pressed against Kodak LR 115 cellulose nitrate detectors, and neutron irradiated. A, Mechanical defects on the detector (dashed areas on *c*); Ca, Commissura anterior; CC, corpus callosum; Cd, nucleus caudatus; Ce, cerebellum; Cinf, inferior colliculus; Cort, neocortex; CS, superior colliculus; FD, fascia dentata; FH, fimbria hippocampi; Fx, columna fornicis; Hip, hippocampus; Hyp, hypothalamus; g, grana; Put, putamen; Th, thalamus; Vl, ventriculus lateralis.

Figure 1 shows our results. The detectors show a certain number of defects seen as dark spots (especially at the level of the midbrain) or scratches (on the cerebellum); however, the different brain substructures are easily recognisable. The hippocampus, fascia dentata, nucleus caudatus, hypothalamus and neocortex are the substructures most heavily loaded with lithium. The cerebellum is also rich in lithium, except for the white matter inside the lobes. At the level of the putamen, careful observation shows lithium-rich striae corresponding approximately to the striae of grey matter. The fimbria hippocampi, superior colliculus and inferior colliculus have moderate lithium concentrations, whereas the thalamus has a rather small lithium content. Even smaller lithium concentrations are found in the corpus callosum, commissura anterior, columna fornicis and the cavity of the ventriculus lateralis. Clearly, the areas which achieve the most integrated operations for the control of motricity, behaviour and emotional attitudes are also those with the highest lithium concentrations. Therefore, in future work, it might be interesting to compare the relative distribution of lithium in the brain of control animals with that in the brain of animals subjected to various stresses, drugs or surgical operations known to affect sensory representation, motricity or behaviour. The resolving power of our method is already good enough to attempt such studies at the histological level. Moreover, as the theoretical limit of the resolving power is estimated²³ to be about 20 Å, further progress in the technology of track detection should eventually allow us to extend the use of the method to the cytological level.

The accumulation of lithium by the hypothalamus can be discussed in relation to the neuroendocrine properties of that area. We have indicated previously²⁰ that another endocrine gland, the hypophysis, also exhibited a high lithium concentration after treatment of the animals with lithium. Moreover, recent data obtained with Vaudry and Tonon (unpublished) indicate that the release of α -melanocyte-stimulating hormone by frog hypophysis is severely inhibited by lithium. This interference with neuroendocrine regulation might have significance in the whole-body effects of lithium.

Figures 2 and 3 are enlarged from Fig. 1, at the level of the cerebellum and the hippocampus. Although there is not a direct law of proportionality, the areas which accumulate much lithium are most generally those with the largest densities of neurone bodies. The reason is perhaps that the distribution of lithium is determined by cell water content at the various areas of the brain; thus, the nerve trunks, being highly lipid, would fail to reveal much lithium uptake. Whatever the reason, this result at least indicates that lithium effects on the brain might originate from the perturbation of the metabolism of some nerve cells, rather than from a direct effect on the process of nervous conduction. For instance, lithium could interact with the biosynthesis of some important proteins in the nervous system. Indeed, in a variety of other cases (sea-urchin embryology²⁴, aging processes^{25,26}, growth²⁷ and bud precedence²⁸ in plants), lithium affects the biosynthesis of some specific proteins, probably at the level of transcription²⁴, without necessarily modifying the bulk of the other protein biosynthesis^{26,27}.

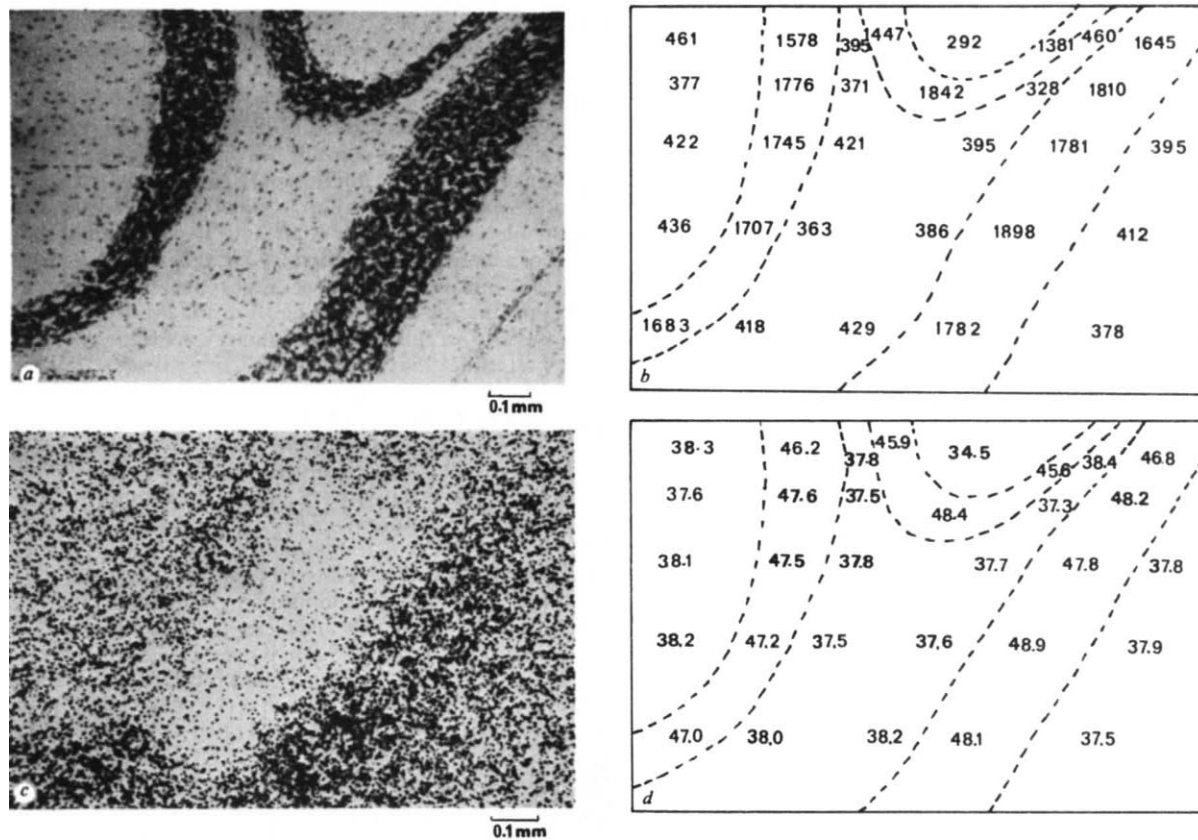


Fig. 2 Enlargement of Fig. 1, at the level of the cerebellum. *a*, Histological preparation; *b*, corresponding estimation of the local densities of cellular nuclei (nuclei per μg); *c*, photograph of the corresponding detector; *d*, estimation of the corresponding local concentrations of lithium (pequiv. per μg).

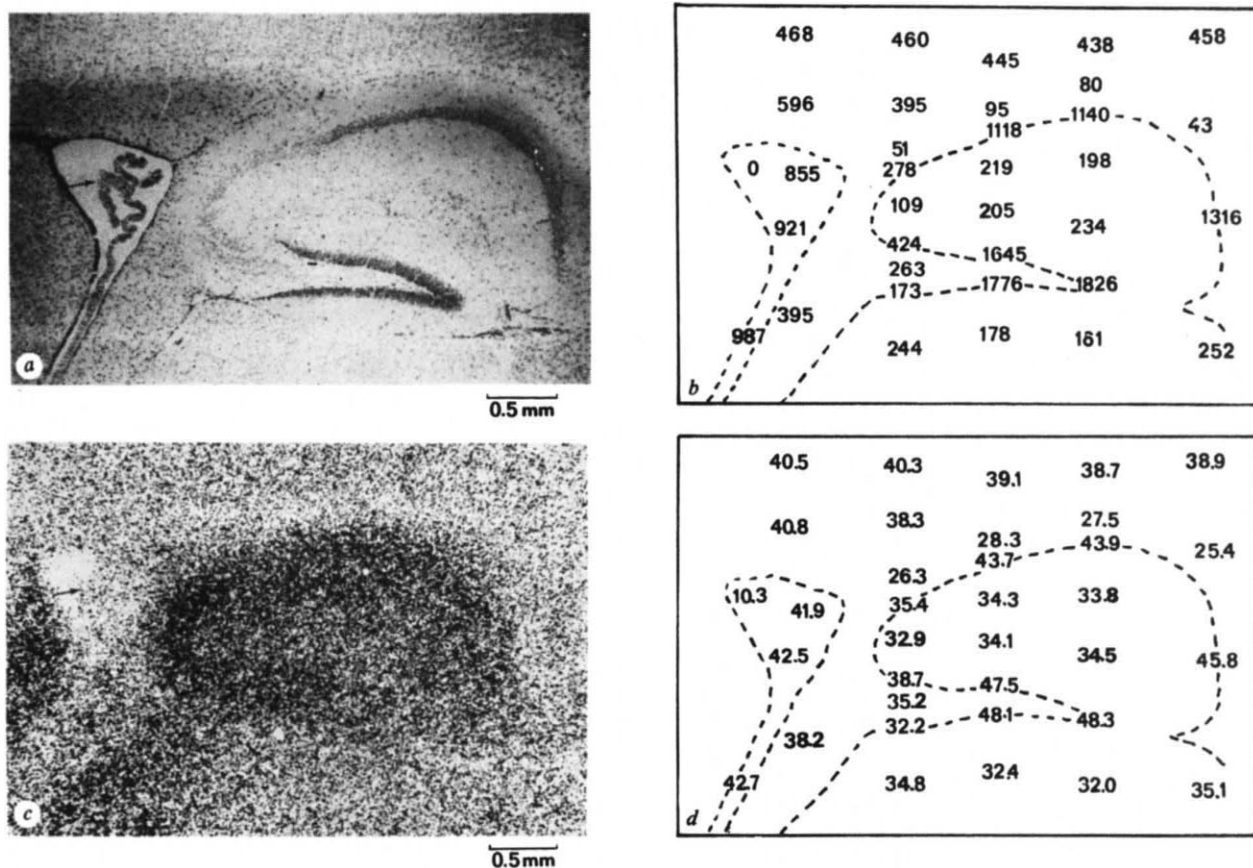


Fig. 3 Enlargement of Fig. 1, at the level of the hippocampus. Same significance of *a*–*d* as in Fig. 2. To appreciate the resolving power of the method, note the correspondence between the histological structures on the section and the distribution of tracks on the detector, especially at the level of the black arrow.

The nuclear track technique can be extended to study the distribution of elements other than lithium. Our group and others have already begun to use it for studying the distribution of boron in plants^{29,30} or in semiconducting alloys³¹, and the labelling of oxygen³² by stable isotope ¹⁷O for the location of catecholamines in the rat brain³³.

This work was supported by grants from the CNRS (L.A. 203 and A.T.P. 'Temps' and 'Petits mammifères') and from the DGRST (77 7 1272). The irradiations were carried out by Dr Stampfer at the reactor of the University Louis Pasteur, Strasbourg, France. We acknowledge the help of Dr Durham in the final preparation of the manuscript.

Received 18 June; accepted 13 November 1979.

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Parasite-induced 'nonspecific' IgE does not protect against allergic reactions

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High levels of immunoglobulin E (IgE) are produced in animals, including man, infected with helminth parasites^{1,2}. Such infection results not only in the production of parasite-specific IgE but also, through polyclonal stimulation of IgE-B cells, in the production of a large excess of parasitic nonspecific IgE³. IgE antibodies mediate allergic reactions by binding to receptors on the plasma membrane of mast cells, which are then said to be sensitised; subsequent attachment of antigen to the membrane-bound antibody causes cross-linking of the receptors leading to the release of histamine and other active substances⁴. Mast cells

can be passively sensitised both *in vivo* and *in vitro* and passive sensitisation with a specific IgE antibody may be inhibited by prior saturation of IgE receptors with an excess of 'nonspecific' IgE^{5,6}. In this way, helminth-infected animals become refractory to passive sensitisation, as do animals bearing IgE myelomas, because of the pre-emption of mast-cell receptors by endogenous IgE^{7,8}. These findings gave rise to two related ideas: first, that it might be possible to ameliorate allergic diseases by passively administering, or by actively stimulating the production of a large amount of 'irrelevant' IgE; and second, that a high parasite-induced IgE level might protect against unrelated allergies by means of competition for mast cell receptors^{6,7,9}. However, searches for epidemiological evidence of such a protective effect have had conflicting results^{10–21}. We have examined this question using egg-albumin (EA)-hypersensitive rats infected with *Nippostrongylus brasiliensis* and now show that 'nonspecific' IgE induced by helminth infection, although having the capacity to block passive sensitisation, does not inhibit hypersensitivity reactions mediated by an actively produced IgE antibody.

Hooded Lister rats immunised with 10 µg of EA and the adjuvant *Bordetella pertussis*, produce IgE antibodies to EA from day 10 after immunisation. Immediate hypersensitivity reactions can then be induced in these rats by an antigen challenge; intradermal injection of EA causes an immediate wheal and flare skin reaction and intravenous injection of the antigen leads to anaphylactic shock. This state of hypersensitivity persists, as does IgE antibody production, for at least 80 days after immunisation²². Although, in theory, other immunological mechanisms can have a role in such allergic reactions, their contribution in the present model is likely to be minimal. IgG2a (short-term sensitising) antibodies cannot be detected in rats immunised with EA by this technique and the level of other antibodies also remains very low.

In a preliminary experiment we attempted to displace actively produced IgE from mast cells by infusing EA-immunised rats with serum from *N. brasiliensis*-infected rats having a very high IgE level: thus, rats were injected daily for 1 week with 5 ml serum containing 140 µg ml⁻¹ total IgE which can be estimated to yield a serum level of 30–40 µg ml⁻¹ in the recipient. The severity of allergic reactions to antigen challenge was not diminished by this treatment.

To create optimum conditions for the demonstration of an allergy-protective effect, we carried out an experiment in which EA-hypersensitive rats were infected with *N. brasiliensis* to stimulate prolonged active synthesis of high levels of irrelevant IgE. In rats infected with this parasite the level of total serum IgE rises from less than 1 µg ml⁻¹ before infection to up to 400 µg ml⁻¹ by the 12th day after infection³. The level remains elevated for many weeks and can be boosted by re-infection²².

The design of the experiment outlined in Table 1 was based on the supposition that an observable blocking effect of nonspecific over specific IgE, in an animal actively producing both, might depend not only on the relative proportions but also on the order of production of the two. Groups of rats were therefore infected with *N. brasiliensis* 20 days before, simultaneously with or 20 days after immunisation with EA. Control groups were immunised but not infected. On various occasions after these treatments the total and antibody IgE status of the rats were assessed and susceptibility to hypersensitivity reactions was determined by antigen challenge.

Table 1 shows the results obtained on the first of these occasions, which is also representative of results obtained later in the experiment. Essentially, we were unable to induce any observable protection either in terms of skin test reactivity or susceptibility to anaphylactic shock by arranging for the occupation of mast-cell receptors with 'nonspecific' IgE whether before, during or after the synthesis of EA-specific IgE. That mast-cell receptors were indeed saturated or otherwise unavailable in the infected animals was confirmed by the finding that these animals, unlike uninfected control rats, could not be effectively sensitised for a passive cutaneous anaphylaxis (PCA)

Table 1 Effect of nonspecific IgE induced by *N. brasiliensis* (Nb) infection on the severity of reactions to egg-albumin (EA) challenge in EA-hypersensitive rats

Group	Immunisation and infection*		Circulating IgE on day 32†			Hypersensitive reactions on day 34‡	
	Day 0	Day 20	Total IgE ($\mu\text{g ml}^{-1}$)	EA-IgE PCA titre	% Rats with detectable circulating EA-IgE	Skin test reaction diameter (mm)	Anaphylaxis score (4 individual rats)
1	Nb	EA	44.6 $\pm$ 4.3	17.1	87.5	13.6 $\pm$ 0.4	4,4,4,2
2	—	EA	0.54 $\pm$ 0.06	12.9	87.5	12.2 $\pm$ 1.2	3,2,0,4
3	EA	Nb	113 $\pm$ 9.8	152.0	100.0	14.6 $\pm$ 1.0	3,3,4,4
4	EA	—	0.65 $\pm$ 0.1	21.1	87.5	12.8 $\pm$ 0.8	2,3,4,3
5	EA + Nb	—	31.5 $\pm$ 2.4	22.6	25.0	11.2 $\pm$ 2.4	2,1,0,3
				(54)	(100)		
6	—	—	0.48 $\pm$ 0.08	0	0	all <5	0,0,0,0

* Outbred female hooded Lister rats (Animal Suppliers) were immunised with EA by intraperitoneal injection of 10 μg EA (Sigma grade V) in 0.15 M NaCl, together with a suspension of 10^{10} heat-killed *B. pertussis*. *N. brasiliensis* infection was by subcutaneous inoculation of 4,000 infective larvae. The method of culture of the parasite has been described previously²⁹. On various occasions after the indicated treatments, eight rats from each group were bled to obtain serum for the determination of total and antibody IgE and 2 days later were challenged with EA and the resulting hypersensitivity reactions assessed. This sequence of bleeding and challenge was carried out on days 32–34, 56–58 and 78–80. The rats of group 3 were re-infected on days 43 and 63.

† Total serum IgE was estimated by a paper radioimmunosorbent test (PRIST)³⁰. Results shown are the mean $\pm$ s.e. of values from eight rats. Specific IgE antibodies to EA were estimated by the passive cutaneous anaphylaxis (PCA) technique³¹ as previously described³. Results shown are the geometric mean PCA titre of animals with a detectable response. To detect IgE antibody in the sera of group 5, the assays were subsequently repeated using the PRIST²⁵ technique the results of which, in units ml^{-1} , are shown in parentheses. The reason for the use of this technique is described in the text.

‡ Hypersensitivity reactions were elicited as follows. Each of four rats received four intradermal injections of 1 μg EA (in 0.05 ml 0.15 M NaCl) on the shaved back followed immediately by intravenous injection of 0.5 ml 1% Evans blue. After 20 min the rats were killed, and the skin test reactions were measured on the inner surface of the skin. The reaction diameters shown are the mean $\pm$ s.e. of the mean values from the four rats. Small reactions only (<5 mm) occurred in unsensitised rats. Anaphylaxis was induced, in an additional four rats per group, by the intravenous injection of 250 μg EA in 0.5 ml of 0.15 M NaCl. Symptoms of shock occurred within seconds and were graded on a scale of severity from 1 to 4 with the latter denoting almost instantaneous death. This dose of antigen caused no reaction in unsensitised rats. The amounts of antigen used were arrived at in a previous dose–effect experiment and were chosen as being sufficiently large to elicit clearly evident reactions in the majority of sensitised animals with minimal or no reactions in normal rats. The object was to operate the system at moderate antigen excess so that the occurrence and size or severity of reactions would be determined by the amount of specific IgE on the mast cell rather than by the amount of antigen injected.

reaction with IgE antibodies to keyhole-limpet haemocyanin.

Note that *N. brasiliensis* infection, in addition to stimulating nonspecific IgE, may enhance or inhibit the EA-IgE response depending on the time between immunisation with EA and infection. A pre-established EA-IgE response is briefly potentiated by the infection^{22,23} as was evident in the rats of group 3 on the first occasion of testing (Table 1), but not later in the experiment. We have previously considered whether this increment in the level of EA-IgE might actually increase the degree of hypersensitivity of the animal to EA but have found that it had no noticeable effect²⁴. In the context of the present experiment, even when the EA-IgE response is potentiated it still comprises a very small proportion of the total IgE³. In rats immunised with EA and simultaneously infected with *N. brasiliensis* (group 5), the production of EA-IgE was inhibited by comparison with rats immunised with EA alone, a phenomenon previously described by Orr and Blair²³ and presumably reflecting a form of antigenic competition. Nevertheless, most of these rats showed marked immediate hypersensitivity to EA, suggesting that EA-IgE antibodies were produced but at levels too low to be detected by the PCA technique in the face of competition by nonspecific IgE for the mast-cell receptors. The subsequent development of a radioimmunoassay for rat IgE antibodies²⁵ (which is not influenced by the presence of irrelevant IgE in the test serum) enabled us to retest these sera and low levels of EA-IgE were indeed detected in them all, as indicated in parentheses.

Our results suggest that as far as the actively sensitised animal is concerned, parasite-induced IgE does not have an appreciable allergy-protective effect. We now believe that the prediction of such an effect has been based on a misleading interpretation of previous passive sensitisation experiments which has failed to consider the turnover of IgE on the mast cell in active sensitisation and the relative proportions of specific and nonspecific IgE which might be expected in helminth infection.

The binding of IgE to its receptor, although of high affinity, is a reversible process (reviewed in ref. 4) and mast-cell-bound and free IgE are in dynamic equilibrium; EA-IgE therefore becomes part of a serum pool of which the constituent molecules are in constant exchange with cell-bound IgE. Therefore, assuming a similar affinity of the molecules for the receptors, EA-IgE, whether produced before, simultaneously with or after nonspecific IgE, will eventually come to be represented on the mast-cell membrane in the same relative proportion as it is found in the serum. Estimates from radioimmunoassay show that in sera from EA-immunised rats infected with *N. brasiliensis* the amount of EA-IgE produced is of the order of 1–100 ng ml^{-1} , with nonspecific IgE forming up to a 10^4 -fold excess. There are approximately 3×10^5 IgE receptors on normal rat mast cells²⁶. Clearly, only a small proportion of these require to be occupied by EA-specific IgE to procure effective sensitisation, as in the parasitised animal most of the receptors will be occupied by IgE molecules with specificity for other antigens. Degranulation studies with the leukocytes of allergic humans show that only a few IgE cross-links, induced either by combination with antigen or anti-IgE antibodies, are required to cause histamine release^{27,28}, and evidently in our system the number of specific IgE molecules bound to the mast cells is such that they are in sufficient proximity to allow the requisite cross-links to be formed.

Thus, the ratio of nonspecific to specific IgE necessary to cause effective 'desensitisation' of mast cells is simply not achieved in helminth infection. Only perhaps in IgE myeloma will concentrations of nonspecific IgE be sufficient to ablate allergic reactions mediated by a normal IgE-antibody response. If allergies are found on balance to be less frequent in helminth-parasitised individuals, the reason is more likely to be found in other mechanisms.

We thank the MRC, the Wellcome Trust and INSERM for support. E.J. is a Locke Research Fellow of the Royal Society.

Received 16 July; accepted 30 November 1979.

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Antibody response to Moloney type C virus-induced tumours

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Animals infected with RNA tumour viruses show an immune response to new virus-induced antigens present on the tumour cell surface. In tumours induced by murine leukaemia virus (MuLV), serologically detected cell membrane antigens have been placed into two major groups; the Gross cell-surface antigen(s), GCSA and G_{IX} , are associated with Gross leukaemia virus infection¹ and the FMR antigen(s), associated with infection by Friend, Moloney or Rauscher type C viruses². There is good evidence that some of these antigens are related to the structural proteins of the virus. The G_{IX} antigen is found on the viral envelope protein, gp70 (refs 3–5) and the GCSA antigen is on the p30 protein which is present in the membrane as part of the glycosylated 'gag' polyprotein(s)^{6–8}. It is not certain whether the FMR antigen(s) is found on membrane-located viral structural precursors or polyproteins, or whether it is present on a tumour-associated protein. Several antigens of this latter type have been described. For example, the Moloney leukaemia virus-induced cell-surface antigen (MCSA) is thought to be a non-viral protein associated with 'gag' products, p30, p15 and p12 on the membranes of Moloney lymphoma cells^{9,10}. MCSA and the antigens on cells infected by Abelson MuLV¹¹ may be very specific cellular antigens associated with Moloney MuLV infection. There has also been much interest in whether the *src* gene product is present in the membrane of sarcoma virus-induced tumours, although it is thought not to be

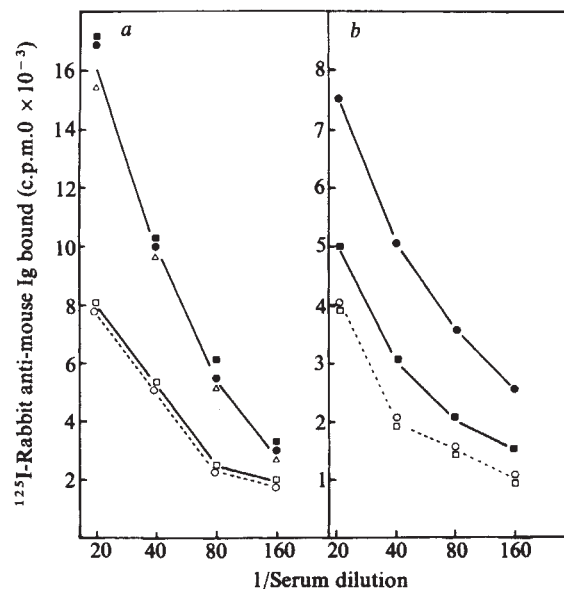


Fig. 1 Specificity of Moloney MuSV serum for type C virus-infected cells using cell monolayer radioimmunoassay. *a*, Moloney MuSV serum was reacted with secondary cultures of fibroblasts from Teylors Own (ICRF) mice, TO cells (□—□); MSC cells (●—●); TO cells infected 7 days previously with Moloney MuSV/MuLV virus contained in MSC supernatants (△—△); TO cells infected with sucrose banded Moloney MuSV/MuLV virus (■—■). Control BALB/c serum reacted with all cell preparations in a similar way (○—○). *b*, Comparison of binding of Moloney MuSV serum with MSC cells (●—●) and AKR-N3T3 cells (■—■) and of binding of control BALB/c serum with MSC cells (○—○) and AKR-N3T3 cells (□—□). Samples were done in triplicate and standard deviations were always less than 10%.

present on the external membrane surface¹². A more controversial point is whether there are other virus-induced cell-surface antigens (CSA) which may serve as targets for an effective immune response to viral tumours¹³. We have previously found¹⁴ that serum from BALB/c mice with regressed Moloney virus-induced fibrosarcomas contained specificities only for Moloney viral proteins and not for any tumour-associated protein like MCSA. We have now identified the viral proteins with which the Moloney MuSV antisera react. Using a syngeneic system of immunisation with freshly induced Moloney sarcoma tumours, we show that the antibody response is directed only at viral structural antigens and that the major part of the response is to antigens on p30 protein probably present as part of a precursor polyprotein on the tumour cell surface.

The antisera used to describe several of the previously mentioned antigens have been obtained after an intensive immunisation schedule. By examining the response of BALB/c mice to regressing Moloney tumours which were induced by Moloney MuSV/MuLV virus rather than by tumour cells maintained in tissue culture, we hoped to obtain a closer approximation of immune responsiveness to a natural infection with virus. Thus, tumours raised in newborn mice by injection of purified Moloney MuSV/MuLV virus were subsequently injected intramuscularly into the flank of each of 20, 4-week-old BALB/c male mice. The resulting tumours regressed within 2 weeks. The serum used in this study, which will be called 'Moloney MuSV serum', represents pooled sera collected at the peak of the antibody response to tumour cells that occurred 18 days after tumour inoculation. A control group of sibling mice was maintained and bled coincidentally with the tumour-bearing mice. The sera were adsorbed with BALB/c embryo fibroblasts before use.

In a cell monolayer radioimmunoassay¹⁴, the Moloney MuSV serum binds to murine TO fibroblasts infected 1 week previously with Moloney virus from two different sources but does not bind

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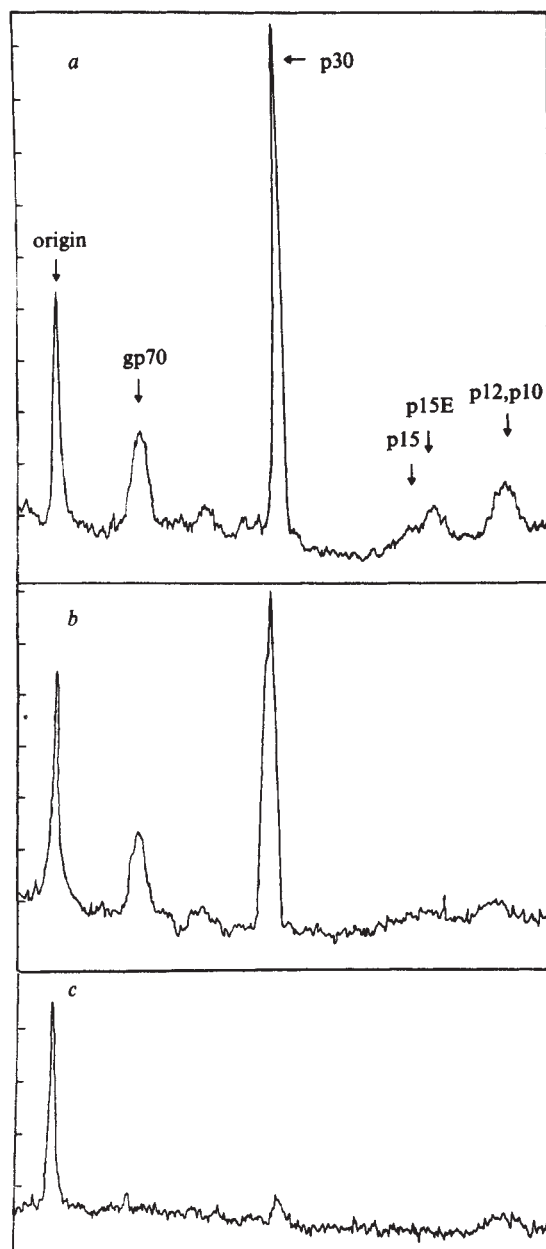


Fig. 2 Scanning densitometer tracings of an autoradiogram of 12% SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ^3H -leucine-labelled Moloney MuSV/MuLV. MSC cells were labelled overnight with radio-labelled virus purified from the supernatant by double banding in a sucrose density gradient and disrupted by freeze-thawing 10 times. Samples of virus containing 50,000 c.p.m. were reacted for 3 h with the appropriate antisera on ice. NP-40 was then added to a final concentration of 1% and the incubation was continued at 4 °C for 16 h. *Staphylococcus aureus* (formaldehyde fixed)²⁹ was then added and the incubation was continued for 1 h at 4 °C. The *Staphylococcus* A-IgG-antigen complexes were removed by low-speed centrifugation, the resulting pellets were then washed (resuspended and centrifuged) with 0.01 M Tris, pH 7.4, followed by 1% sodium deoxycholate, and finally 0.01 M Tris, pH 7.4. The samples were dissolved in SDS-sample buffer and applied to an SDS gel as previously described³⁰. Adsorption of Moloney MuSV serum with cells was done by incubation of serum and cells at a ratio of 1:4 for 30 min on ice. Samples of ^3H -leucine-labelled Moloney MuSV/MuLV were precipitated by Moloney MuSV serum (a), antiserum adsorbed with AKR-N3T3 cells (b) and antiserum adsorbed with MSC cells (c). Standards used for calculating molecular weight were a sample of ^3H -leucine-labelled Moloney MuSV/MuLV and β -galactosidase (130,000), phosphorylase a (94,000), lactoperoxidase (80,000), catalase (60,000), alcohol dehydrogenase (41,000), carbonic anhydrase (29,000) and myoglobin (17,200) (not shown).

to any significant degree to uninfected fibroblasts (Fig. 1a). Control serum showed low levels of binding to both virus-infected and uninfected cells. Similarly, the Moloney MuSV serum bound to rat fibroblasts infected with Moloney virus, but not to uninfected rat fibroblasts (data not shown). As shown in Fig. 1b, the Moloney MuSV serum reacts with the MSC cell line¹⁵, which is derived from a Moloney virus-induced sarcoma, and only to a small degree with Gross virus-infected N3T3 cells, called AKR-N3T3 cells¹⁶; this suggests that the Moloney MuSV serum reacts chiefly with type-specific determinants induced by virus on Moloney virus-infected cells.

We have previously shown that the activity of this type of serum can be completely removed by adsorption of the serum with sucrose gradient Moloney MuSV/MuLV virus purified by sucrose gradient banding¹⁴. This has been confirmed in the present study and suggests that the major specificities in the Moloney MuSV serum are directed against antigens present on viral polypeptides expressed on the cell membrane. To assess which viral proteins were being recognised, disrupted ^3H -leucine-labelled Moloney MuSV/MuLV virus shed by the MSC cell line was precipitated with the Moloney MuSV serum. The Moloney MuSV serum precipitated predominantly p30 and gp70 proteins (Fig. 2a). Proteins coincident with p15(E) and p10/p12/p12(E) are precipitated to a minor degree and are therefore only faintly apparent in Fig. 2a. When the Moloney MuSV serum was adsorbed with Gross virus-infected AKR-N3T3 cells, the titre of antibodies reacting with p30 or gp70 was only slightly reduced (Fig. 2b), but when a similar adsorption was done with Moloney virus-infected MSC cells these specificities were completely removed (Fig. 2c). Control serum from unimmunised mice did not precipitate any viral proteins (data not shown). Therefore, we conclude that Moloney MuSV-induced fibrosarcomas induce antibody with specificities for Moloney type-specific determinants on p30 and gp70 proteins and that both are candidates for FMR antigens.

Adsorption of Moloney MuSV serum with Gross virus-infected AKR-N3T3 cells did remove some anti-p30 antibody and low titre antibodies reacting with the lower molecular weight viral proteins (Fig. 2b). These antibodies, as has been suggested by others¹⁷⁻¹⁹, may be responsible for the cross-reactive or group specificity shared between the Moloney and Gross infected cells (Fig. 1b).

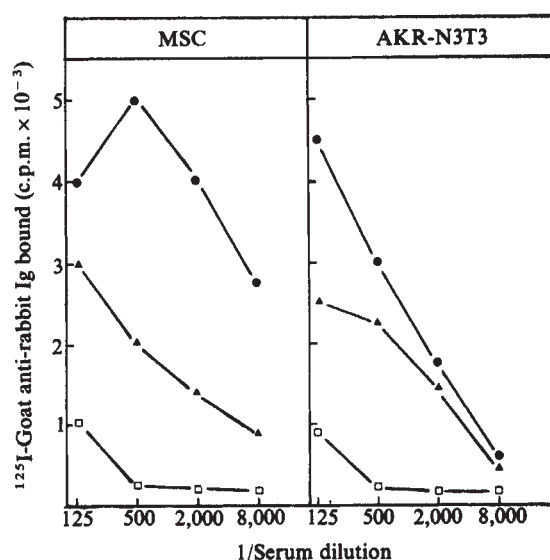


Fig. 3 Moloney MuSV/MuLV shedding MSC cells and the Gross MuLV shedding AKR-N3T3 cells were examined for expression of membrane type C viral antigens using a cell monolayer radioimmunoassay¹⁴. The cells were reacted with the following antisera to type C viral proteins¹⁴: rabbit anti-gp70 (●), rabbit anti-p30 (▲) and a control normal rabbit serum (□).

It was possible that AKR-N3T3 cells failed to adsorb p30 and gp70 antibodies from the Moloney MuSV serum because very little of these proteins were present on the membranes of the cells. To investigate this, rabbit anti-p30 and anti-gp70 reacting chiefly with shared or group-specific antigens¹⁴ were titrated on both MSC cells and AKR-N3T3 cells. The titration profiles (Fig. 3a, b) show that both cell types have easily detectable amounts of both gp70 and p30 on their membranes. It is proposed that AKR-N3T3 cells fail to adsorb antiviral specificities from Moloney MuSV serum (Fig. 2b) because these antibodies are directed against Moloney virus type-specific determinants on both gp70 and p30 proteins.

FMR antisera have been shown to exhibit virus-neutralising activity and to contain antibodies which are cytotoxic for FMR-infected cells.²⁰ It has been reported that the cytotoxicity can be removed by adsorption of the antisera with an internal virion protein thought to be p15 (ref. 21). More recently, Nowinski and coworkers have provided evidence associating FMR antigenicity with the envelope protein, gp70 (ref. 22). In the present study, we have also found type-specific responses to gp70 protein but, in addition, we found higher titres of antibody directed against type-specific antigens on p30 protein. The finding of an immunogenic type-specific p30 antigen(s) as a possible component of the FMR antigen(s), suggests that this antigen is analogous to GCSA antigen induced by Gross virus infection, which is present on p30 as part of membrane bound 'gag' polypeptide. We do not know the molecular nature of the Moloney virus cell membrane p30. Many other species such as rats, cats, hamsters, rabbits and higher primates make antibody to p30 (refs 19, 23, 24) in response to type C virus infection. Mice have formerly been thought to be tolerant to p30 protein²⁵⁻²⁷ unless immunised across strain barriers, as was done to raise the GCSA antisera¹. Our study indicates for the first time that mice can mount a good response to p30 when immunised syngeneically and that the responses to G_{IX} and GCSA antigens on Gross virus tumours^{1,28} and the FMR antigen on Friend, Moloney and Rauscher virus tumours are very similar in that both are type-specific responses to gp70 and p30, and not, in general, to other tumour-associated proteins.

We thank Dr Jennifer Harvey for Moloney MuSV/MuLV virus and for her help in inducing Moloney tumours, Ms Susan Crowhurst for technical assistance, and Mike Fried, Dick Knight and N. A. Mitchison for reading the manuscript. N.H. is supported by the Leukaemia Research Fund.

Received 12 July; accepted 9 November 1979.

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Anti-idiotypic antibodies in a patient with a functioning renal graft

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A central problem in improving the success of renal transplantation in humans is to discover how to induce in the recipient an active immunological unresponsiveness to the donor's alloantigens¹⁻³. There is increasing evidence that immunological unresponsiveness can be induced by either suppressor T cells^{4,5} or antibodies directed against the T-cell receptor, that is, anti-idiotypic antibodies^{6,7}. In our institutes, there are now six successful renal recipients. One of these, CD-S5, is a 32 year old man, HLA tissue-typed as Aw31, Bw39, Bw54 and DRw4. He received a second kidney transplant from a cadaveric donor having HLA-A2 and B5 on 21 August, 1975, after the first transplant from his brother with Aw26 and Bw40 had been rejected. The stimulation index of the mixed lymphocyte reaction (MLR) between patient and first donor was 11.9 but MLR could not be tested in the second grafting. One year after the second graft, immunosuppressive treatment with azathioprine was withdrawn because of drug toxic hepatitis. In spite of the administration of only prednisolone at 10 mg per day for the past 3 yr, the patient's creatinine clearance has remained at 70 ml min⁻¹. We proposed that the long survival of the renal graft might be due to an immunological unresponsiveness induced by the host immunoregulatory mechanism of this patient. To test this possibility, we have investigated the patient's serum for factor(s) capable of suppressing the host immune response. We report here our finding that the patient's serum contained an antibody against a T-cell receptor for a certain MLR, which was capable of specifically inhibiting a certain MLR.

First, we carried out an MLR inhibition test between recipient and donor using the recipient's serum. However, the recipient had received his graft from a cadaveric donor and the responsiveness of his lymphocytes in the MLR was found to be very weak, probably because they had already been inactivated, either by the host regulatory mechanism or by the immunosuppressive drug. A similar HLA-mismatched combination from a third individual was then attempted. As the HLA-B locus is more closely linked to the HLA-D locus (the MLR locus) than HLA-A, a combination of responder T.M. (A2, A11) (Bw15,) (DRw4,) and stimulator R.H. (A2, Aw26) (B5,) (DRw2,) cells was selected from healthy individuals who had the same mismatched HLA-B antigen B5 as was found between recipient and cadaveric donor. B5 antigen has recently been split into two antigens, Bw51 and Bw52, and B5 of the stimulator R.H. was later defined as Bw52. Using this combination, the patient's serum was subjected to the MLR inhibition test by its addition to the mixed lymphocyte culture. As shown in Table 1, the patient's serum clearly inhibited the MLR of this combination. The characteristics of the inhibitory factor in the patient's serum were then investigated to determine whether or not it was an antibody. For this, the patient's serum was fractionated by Sephadex G-200 column chromatography with borate-buffered saline, pH 8.0. Fractions of 19S, 7S, 4S and <4S, after being concentrated to the original volume by negative pressure dialysis, were each tested for their inhibitory activity in the MLR. Inhibitory activity was found mainly in the 7S fraction, and was

Table 1 The effect of the renal recipient's serum fraction on the MLR of the selected third-party combination

Serum fraction added to the MLR	³ H-Thymidine incorporation by T.M. cells against R.H. (mean c.p.m. ± s.d.)
—	1,384 ± 252*
Not added	16,856 ± 1,234
Whole serum	1,480 ± 396
19S fraction	11,799 ± 1,099
7S fraction	3,371 ± 469
4S fraction	5,900 ± 899
<4S fraction	15,170 ± 769
7S fraction absorbed with rabbit anti-human IgG ab.	15,822 ± 1,177

The serum of the renal recipient was fractionated by elution on Sephadex G-200 column chromatography (2.6 × 100 cm) with borate-buffered saline, pH 8.0. As the inhibitory activity was mainly found in the 7S fraction, it was absorbed with purified rabbit antibodies directed against human IgG at room temperature for 1 h and then at 4 °C overnight at optimal ratio. Ten per cent of unabsorbed 7S fraction or absorbed 7S fraction was added to the culture medium of the selected MLR which had the same mismatched HLA-B antigen, B5, as found between recipient and cadaveric donor. Phenotype of responder T.M. is (A2, A11) (B15,) (DRw4,) and that of stimulator R.H. is (A2, Aw26) (Bw52,) (DRw2,). For MLR, 1 × 10⁵ lymphocytes, prepared from defibrinated peripheral blood, were cultured with 1 × 10⁵ 2,000-rad irradiated stimulating cells for 5 days in microtissue culture plates (Nunc N-1182, Roskilde) using RPMI-1640 medium (GIBCO) with 10% heat-inactivated human serum. Sixteen hours before collection, 0.5 µCi of ³H-thymidine was added to each well and its incorporation measured with a liquid scintillation counter. Values are expressed as mean c.p.m. ± s.d. of triplicate samples.

* The value shows ³H-thymidine incorporation by responding cells without stimulating cells.

completely removed by the absorption of IgG from the fraction using purified rabbit antibodies against human IgG at an optimal ratio (Table 1). This indicated that the inhibitory effect on the MLR is mediated by IgG antibodies. The serum IgG fraction was then isolated on DEAE-cellulose column chromatography by elution with 0.005 M phosphate buffer, pH 8.0, and tested for specificity of the MLR inhibition using various responder-stimulator cell combinations (Table 2). Treatment of responder cells (2 × 10⁶ cells) with 0.5 ml of the antibody fraction

(3 µg ml⁻¹) selectively inhibited the MLR of only those combinations with the same mismatched HLA-B antigen B5 as found in the combination of recipient and cadaveric donor. Also, the IgG fraction did not inhibit the MLR of the responder cell S.S., which differs from T.M., against any stimulating cells. Furthermore, the treatment of the stimulating cell R.H. with the antibody fraction had no effect on the response of untreated responder T.M. cell against the treated R.H. cell (Table 2), indicating that the inhibition is specific for very restricted combinations and is not mediated by antibody directed against common surface antigens of either the responder or the stimulator cell. In addition, the inhibitory effect on the MLR was directed against the responder cells, not against the stimulator cells.

The specificity of the inhibition by the antibody fraction was confirmed by an absorption study. When the antibody fraction (3 µg protein ml⁻¹) was absorbed three times with the responding cell T.M. (1 × 10⁷) the inhibitory activity was markedly reduced, whereas absorption with an equal number of stimulator cell I.M. failed to remove the inhibitory activity (Table 3). Therefore, this restricted inhibitory effect on the MLR of specific pairs indicates that the antibody found in the patient's serum is directed against the T-cell receptor initiating MLR of the responding cell.

The nature of the T-cell receptor has been investigated in several laboratories^{8,9}. Studies using anti-idiotypic antibody provide evidence that the T cell has receptors sharing idiotypes with the B cell for T-cell mediated immune reactions. Binz *et al.*¹⁰ have already reported that the MLR, which is thought to be a T-cell mediated immune reaction¹¹, can be inhibited by an anti-idiotypic antibody directed against alloantibodies or T-cell receptors for alloantigens. An anti-idiotypic antibody has also been shown to compete with the corresponding alloantigen for binding to MLR-activated T-cell blasts¹², indicating that the inhibition of the MLR is mediated by the binding of anti-idiotypic antibodies to the antigen binding site of the T-cell receptor. The present finding that the inhibitory activity of the MLR in the recipient's serum is completely removed by absorption with purified rabbit antibodies directed against human IgG shows that the activity is associated with IgG antibody and directed against responder cells, but not stimulator cells. Moreover, the treatment of responder cell T.M. with the IgG antibody completely inhibited only the response against two types of stimulator cells, those which share Bw52 and DRw2,

Table 2 The effect of serum IgG antibody fraction from the renal transplant patient CD-S5 on various third-party MLR

Phenotype of responder*	Phenotype of stimulator*	³ H-Thymidine incorporation (c.p.m. ± s.d.)		
		Untreated	Treated responder	Treated stimulator
T.M. (2, 11) (15,) (4,)	—	1,288 ± 243	1,175 ± 181	—
	R.H. (2, 26) (52,) (2,)	16,392 ± 732	1,118 ± 220	20,220 ± 2,225
	I.M. (24,) (15, 52) (2,)	14,373 ± 2,883	1,969 ± 105	ND
	O.Y. (24,) (51, 54) (4,)	19,295 ± 1,181	22,679 ± 1,637	ND
	K.E. (11, 26) (12,) ()	15,575 ± 1,657	19,756 ± 786	ND
	M.T. (2, 26) (40,) ()	22,317 ± 773	21,988 ± 832	ND
	D.N. (2, 26) (40,) (4,)	17,016 ± 908	15,054 ± 1,696	ND
	M.Y. (2, 26) (15, 35) (4,)	12,475 ± 760	10,699 ± 2,831	ND
	M.N. (24, 31) (51, 52) (4,)	10,185 ± 1,042	12,903 ± 2,574	ND
	J.H. (11, 24) (13, 52) (2, 5)	15,394 ± 906	11,155 ± 936	ND
	T.H. (11, 24) (7, 15) (1, 4)	10,703 ± 174	12,387 ± 302	ND
	K.S. (2, 24) (35, 40) (2,)	19,110 ± 704	21,207 ± 3,155	ND
	S.T. (26, 31) (15, 52) (2,)	15,738 ± 346	11,044 ± 129	ND
	M.K. (2, 24) (15, 52) (2,)	16,858 ± 1,650	11,743 ± 1,520	ND
S.S. (33,) (12, 40) ()	—	1,404 ± 324	—	—
	R.H. (2, 26) (52,) (2,)	11,674 ± 832	13,782 ± 603	ND
	I.M. (24,) (15, 52) (2,)	9,526 ± 307	8,458 ± 910	ND
	T.M. (2, 11) (15,) (4,)	13,307 ± 627	14,666 ± 674	ND

The IgG fraction was isolated from the patient's serum by DEAE-cellulose column chromatography using 0.005 M phosphate buffer at pH 8.0. 3 × 10⁶ cells of either responder T.M. or stimulator R.H. were treated with 0.2 ml of the IgG antibody fraction (3 µg ml⁻¹) for 30 min at 37 °C and then washed three times with RPMI-1640 medium. After treatment, the responder cells were mixed with 12 different irradiated stimulator cells. In another experiment, R.H. stimulator cells were mixed with untreated responder cells T.M.

* The first parenthesis is for A locus, the second for B locus, and the third for DR locus antigens.

Table 3 Absorption of the inhibitory activity of the antibody fraction with the responder (T.M.) cells but not with the stimulator (I.M.) cells

Phenotype of responder*	Phenotype of stimulator*	³ H-Thymidine incorporation by T.M. cells (mean c.p.m. $\pm$ s.d.) treated with		
		None	Unabsorbed antibody fraction	Antibody fraction absorbed with T.M. / Antibody fraction absorbed with I.M.
T.M. (2, 11) (15,) (4,)	—	986 $\pm$ 264	—	—
	R.H. (2, 26) (52,) (2,)	8,836 $\pm$ 266	423 $\pm$ 178	8,352 $\pm$ 972 / 1,967 $\pm$ 575
	I.M. (24,) (15, 52) (2,)	7,443 $\pm$ 735	773 $\pm$ 115	7,553 $\pm$ 768 / 1,758 $\pm$ 291
	S.S. (33,) (12, 40) (,)	9,603 $\pm$ 428	7,715 $\pm$ 215	8,911 $\pm$ 700 / 8,461 $\pm$ 244

The IgG antibody fraction had an inhibitory effect on the MLR even at a concentration of $3 \mu\text{g ml}^{-1}$. Therefore, 0.5 ml of $3 \mu\text{g ml}^{-1}$ of the IgG antibody fraction was absorbed with either 1×10^7 I.M. stimulator cells or 1×10^7 T.M. responder cells for 30 min at 37°C . The absorption was repeated three times with the same number of fresh cells each time. After absorption, the IgG fraction was tested for its inhibitory effect on the MLR, treating the responder cells T.M. with either unabsorbed or absorbed IgG antibody fraction.

* The first parenthesis is for A locus, the second for B locus and the third for DR locus antigens.

but did not inhibit the response against 11 other types of stimulating cells. This suggests that the antibody is directed against a restricted clone of T cells bearing the MLR antigen of the stimulating cells R.H. and I.M. From this, and observations reported by others, we consider that the patient's serum contains antibody against a T-cell receptor for an MLR antigen, that is, an anti-idiotypic antibody. Genotype for the responder cell T.M. is A2, /A11, Bw15, DRw4; for the stimulating cell R.H., A2, /Aw26, Bw52, DRw2; and for the stimulator cell I.M., Aw24, Bw15, /Aw24, Bw52, DRw2. Phenotype of the cadaveric donor of the patient was (A2,) (B5,). From these combinations, because one haplotype of stimulator R.H. or I.M. is in parts identical to that of the responder T.M., we suggest that this antibody could bind an idio type of the receptor specific for DRw2-associated MLR determinants.

In contrast to our findings, it has been reported that in experimental animals, idiotypes of T cells usually differ between strains, although intrastrain idiotype cross-reactions of T-cell receptors are extremely strong¹³. However, it has been observed that idiotypes are shared in immunoglobulins from genetically different individuals¹⁴. Furthermore, T-cell receptors are generally idiotypically more homogeneous than antibodies^{15,16}. These findings suggest that certain idiotypes are shared by a T-cell clone of T.M. and the renal recipient, which can be activated with DRw2-associated MLR antigen.

As presented by Jerne, the immune network theory claims that the autoimmune response to self-idiotypes can basically act as an immunoregulatory system¹⁷. The passive immunisation with an anti-idiotypic antibody or active immunisation with parental cells of F₁ hybrids induces immunological unresponsiveness in animals against the relevant antigen^{6,18}. This idea seems attractive for clinical transplantation, and it is conceivable that anti-idiotypic antibodies directed against autologous idiotypes on T cells are at least partly responsible for the successful graft survival in the patient not receiving azathioprine.

Received 23 July; accepted 31 October 1979.

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Stimulation of cytolytic T cells by isolated viral peptides and HN protein coupled to agarose beads

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Sendai virus-infected mouse cells can be lysed by mouse cytolytic thymus-dependent lymphocytes (CTL) directed specifically against the infected cells. The CTL is known to recognise the H-2 antigens on the target cells together with structure(s) including at least the two viral surface glycoproteins also found on purified virus^{1–8}. We report here that anti-Sendai CTL can be stimulated *in vitro* by detergent-solubilised viral haemagglutinin-neuraminidase (HN)⁹, either as the isolated protein or coupled to agarose beads. We further show stimulation by the hydrophilic portion of a protein removed from the virus by the protease subtilisin BPN', and we demonstrate that cyanogen bromide- (CNBr-) cleaved viral peptides also produce such stimulation.

For studies with the detergent-solubilised Sendai glycoproteins we made three preparations: (1) a mixture of HN and the fusion protein (F)^{9,10}, denoted HNF, contaminated by a small amount of nucleoprotein (NP)⁹, or a breakdown product of HN¹⁹. (2) cleanly purified F, and (3) and HN fraction separated completely from the F but retaining a trace amount of NP or HN breakdown material (Fig. 1). Since the NP is normally associated with the nucleic acid at the interior of the virus⁹, we assume that this protein is not likely to be a CTL antigen. Thus any small amount of NP in the HN and HNF preparations should not affect their properties in CTL stimulation.

The preparations of HNF, F and HN were either: (1) left untreated, (2) dialysed to remove detergent, or (3) coupled covalently to *N*-hydroxysuccinimide-activated agarose beads at initial concentrations ranging from 25 to 60 μg protein per 0.5–6.5 ml packed beads in a total volume of 3–15 ml. In preliminary experiments, the HNF and HN, either free or bound to beads, were consistently able to stimulate H-2-restricted anti-Sendai CTL *in vitro* from spleen cells of BALB/c(H-2^d) and C57BL/6(H-2^b) mice previously exposed to Sendai virus. The BALB/c CTL were typically able to lyse P815(H-2^d) mastocytoma cells only after Sendai infection and were unable to lyse EL4(H-2^b) leukaemia cells either with or without Sendai infection. In contrast, HNF- and HN-stimulated C57BL/6(H-2^b) CTL specifically lysed infected EL4(H-2^b) but not P815(H-2^d) cells either with or without infection. There did

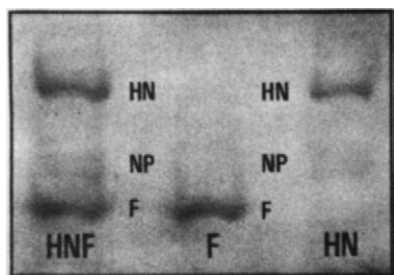
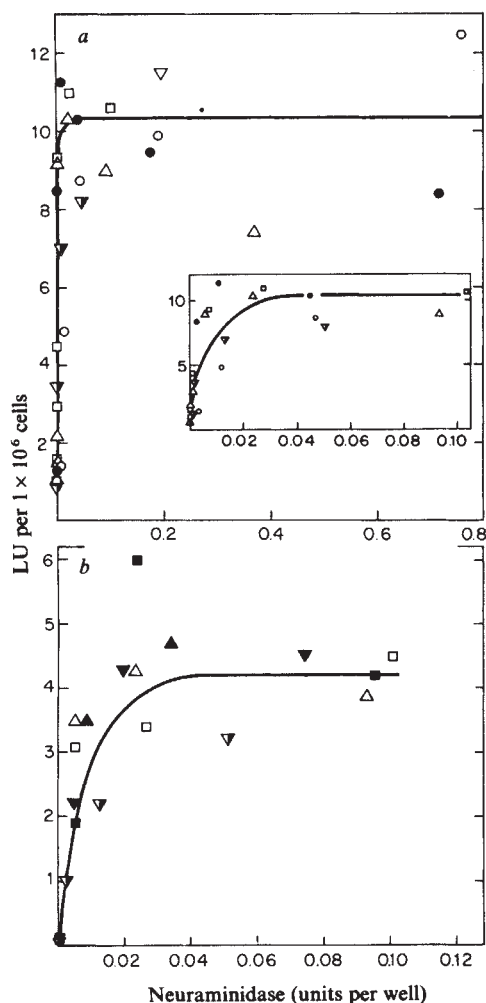


Fig. 1 Preparation of Sendai F, HN and HNF and their presentation to mouse spleen cells for CTL stimulation *in vitro*. SDS-polyacrylamide gel electrophoresis of Sendai protein fractions. Purified Sendai virus was extracted with 0.25% Triton X-100 in 0.01 M sodium phosphate buffer, pH 7.2, at 6.4 mg protein ml⁻¹ for 30 min at room temperature with repeated mixing in a homogeniser. After centrifugation at 200,000g for 1 h (ref. 9), the HNF was contained in the supernatant of the extracted proteins. The yield of HN was 20% as measured by neuraminidase enzyme assay. The HN fraction was HNF material which did not absorb to a diethylaminoethyl-agarose (DEAE-BioGel A, BioRad) column equilibrated with 0.1% Triton X-100 in 0.01 M sodium phosphate buffer, pH 7.2 (ref. 16). The F fraction was HNF material which did not absorb to a hydroxyapatite (BioGel HTP, BioRad) column equilibrated with the same buffer. The yield of both F and HN was over 80% from the two columns. The purified HN had 460 units neuraminidase per μ g protein. Aliquots of 15 μ g HNF, 6 μ g F and 9 μ g HN were loaded onto the 7% gel¹⁷.



tend to be some stimulation of C57BL/6 CTL against uninfected EL4 cells although this stimulation was always less marked than that for the infected cells. These characterisation experiments showed that the fact that the BALB/c CTL did not lyse the Sendai infected EL4 cells was not due to the inability of the infected EL4 cells to act as targets for anti-Sendai CTL. On the contrary, the CTL stimulated by the isolated viral proteins gave the typical H-2 restriction seen for anti-Sendai CTL¹⁻⁸. In view of the more clearcut viral dependence with the BALB/c CTL, further experiments were performed with this system.

Results with the isolated F protein, either free or on agarose beads, were inconsistent, at best showing marginal stimulation; so experiments with the F protein were not pursued further. However, F is in fact an antigen recognised by anti-Sendai CTL since the F and H-2 proteins inserted together into liposomes can stimulate these CTL although the antigenicity seems to be lower than that for HN⁸.

The extent of stimulation by the HN and HNF preparations was closely correlated to the amount of neuraminidase and hence HN in the preparations regardless of whether the antigen was free in a detergent-containing solution, had been removed from the detergent by dialysis, or had been coupled to agarose beads (Fig. 2). The same maximum stimulation was observed with 0.04–0.80 units neuraminidase per well and 130–16,000 beads per well. The minimum amount of HN needed to give maximum stimulation represented enzyme from 30 virus particles per cell. The presence of the extra F in the HNF preparations did not increase stimulation beyond that of HN alone, confirming that the F had little if any stimulatory activity. The

Fig. 2 Dependence of anti-Sendai CTL stimulation on HN concentration. The HNF and HN preparations of Fig. 1 were shaken gently overnight in the cold with *N*-hydroxysuccinimide-activated agarose beads (2×10^6 packed beads ml⁻¹, bead diameter 50–200 μ m, Affigel-10, BioRad) in 0.1 M HEPES buffer, pH 6.5. The covalently coupled beads were then washed with 0.1% Triton X-100 in 0.01 M sodium phosphate buffer to remove unattached protein, and unreacted *N*-hydroxysuccinimide groups were blocked by further shaking in 0.1 M glycine ethyl ester, pH 8.0 in 0.1% Triton X-100 for 1 h. The beads were finally washed repeatedly and suspended in TD buffer (8g NaCl, 0.38g KCl, 0.1 g Na₂HPO₄, 3.0 g Tris-HCl per litre, pH 7.4). (In the descriptions below involving the coupled beads, the concentrations at the beginning of the coupling are indicated in brackets in μ g ml⁻¹ for protein and beads per ml $\times 10^5$ for the Affigel.) From neuraminidase enzyme measurements, the binding of HN to beads for HN(12)-Affigel(3) was 12% to give 4.5 units neuraminidase per 10^5 beads. The corresponding values for HN(5)-Affigel(6) were 29% and 2.8 units, for HN(2)-Affigel(7) were 43% and 1.3 units, for HNF(20)-Affigel(3) were 14% and 6.6 units, for HNF(10)-Affigel(6) were 20% and 2.4 units, and for HNF(4)-Affigel(7) were 51% and 1.9 units. For the stimulation of anti-Sendai CTL, spleen cells ($3-4 \times 10^6$) from BALB/c (H-2^d) mice immunised intraperitoneally several weeks previously with live Sendai virus were added to 16-mm diameter tissue culture wells (Linbro Scientific) together with the additions indicated in 0.5 ml serum-free medium. After 30 min at 37 °C, serum containing medium was added to give 2.5 ml per well at the final composition used previously¹⁸. The CTL in the wells were assayed as described¹⁸ for the ability to release radioactivity from ⁵¹Cr-labelled P815 (H-2^d) cells exposed to an excess of UV-inactivated Sendai virus in a small volume of serum-free medium for 1 h. The lytic units per 10^6 cultured cells (LU per 10^6) were as defined previously¹⁸, except that lysis was measured after 6 h instead of 3 h. *a*, The inset has the same data plotted on an expanded scale. The antigen added to these wells were: the HN preparation as in Fig. 1 in detergent solution (○), the same preparation dialysed extensively against TD buffer to remove the Triton X-100 (●), HN(12)-Affigel(3) (△), HN(5)-Affigel(6) (□), and HN(2)-Affigel(7) (▽). Two control cultures stimulated with an excess of UV-inactivated Sendai virus gave 94 and 176 LU per 10^6 respectively. The inset shows the same data plotted on an expanded scale. The antigen added to these wells were the same HN-Affigel preparations used above (same symbols), plus: HNF(20)-Affigel(3) (▲), HNF(10)-Affigel(6) (■), and HNF(4)-Affigel(7) (▼). Two control cultures similar to the ones used in *a* gave 70 and 119 LU per 10^6 respectively.

anti-Sendai CTL were indeed thymus-dependent because lysis could be reduced more than 99% by treatment with anti-Thy 1.2 antibody and rabbit complement.

Since the haemagglutinin activity of the HN will cause the protein to bind to cell surfaces, it was conceivable that the protein was acting as a lectin in the same way as concanavalin A (Con A), which is known to stimulate CTL nonspecifically¹¹. This possibility was unlikely since HNF beads did not efficiently stimulate anti-H-2^d specific CTL from C57BL/6(H-2^b) mice previously immunised with H-2^d cells, while Con A on beads was able to do so (Fig. 3). Conversely, Con A on beads did stimulate these allospecific CTL but not the anti-Sendai CTL as the HNF beads had done. The ability of bead-bound Con A to stimulate at least one class of T cells is in contrast to a previous report¹² that Con A bound to surfaces could stimulate B but not T cells. It is conceivable that only a small subset of T cells could be stimulated by Con A on beads and that this subset was missed by the earlier report.

The results with the bead-bound antigen suggested that stimulatory antigen need not be presented to spleen cells in the context of a lipid membrane. The ability of antigen coupled to beads to stimulate CTL has also been demonstrated for H-2 alloantigens¹³. Next, we tested for stimulation by fragments of Sendai proteins, beginning with those cleaved from the virus by subtilisin BPN'. In the conditions used, the HN was liberated from the virus as demonstrated by mobility of the neuraminidase activity at a rate corresponding to a molecular weight smaller than bovine serum albumin (molecular weight 68,000) on an agarose gel filtration column (Fig. 4a).

Since our object was to determine the minimum antigenic determinant for anti-Sendai CTL stimulation, we chromatographed the subtilisin-solubilised viral proteins on a Sephadex G-75 column with an exclusion limit of molecular weight 70,000

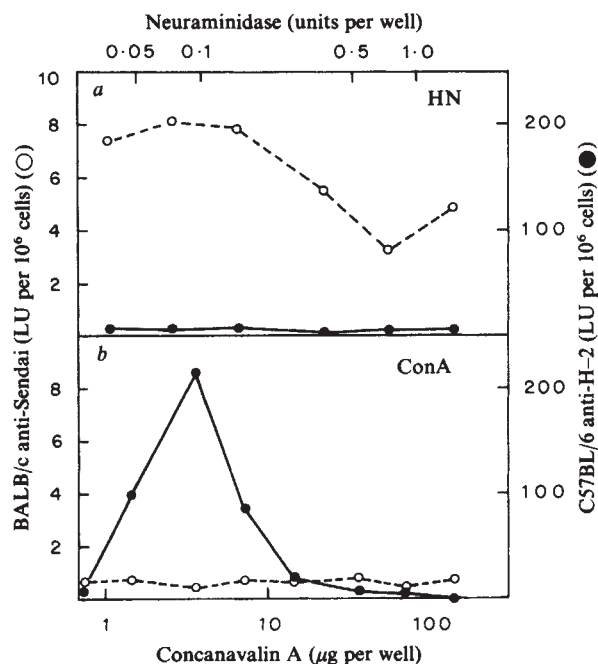


Fig. 3 Specificity of HNF stimulation of CTL. *a*, BALB/c(H-2^d) spleen cells were stimulated with HNF(4)-Affigel(14) at various concentrations and assayed for anti-Sendai CTL using Sendai-infected P815(H-2^d) target cells as described in Fig. 2. Spleen cells from C57BL/6(H-2^b) mice immunised intraperitoneally several weeks previously with 30×10^6 P815(H-2^d) cells were also stimulated *in vitro* as above with HNF(4)-Affigel(14) and were assayed for anti-H-2^d CTL using the same Sendai-infected P815 target cells. The assay was for 3 h instead of 6 h. The results were essentially the same when non-infected P815 target cells were used. *b*, The same conditions were used in the stimulation and assay except that concanavalin A covalently coupled to agarose beads at 15 mg ml^{-1} beads (Miles Labs) was used in place of the HNF(4)-Affigel(14).

and tested the fractions for both neuraminidase activity and CTL stimulation (Fig. 4b). The enzyme activity appeared almost in the excluded volume, indicating that the solubilised HN had a larger apparent molecular weight on this column than on the agarose column. Anti-Sendai CTL stimulatory material was found in the region where the HN protein and smaller materials ran.

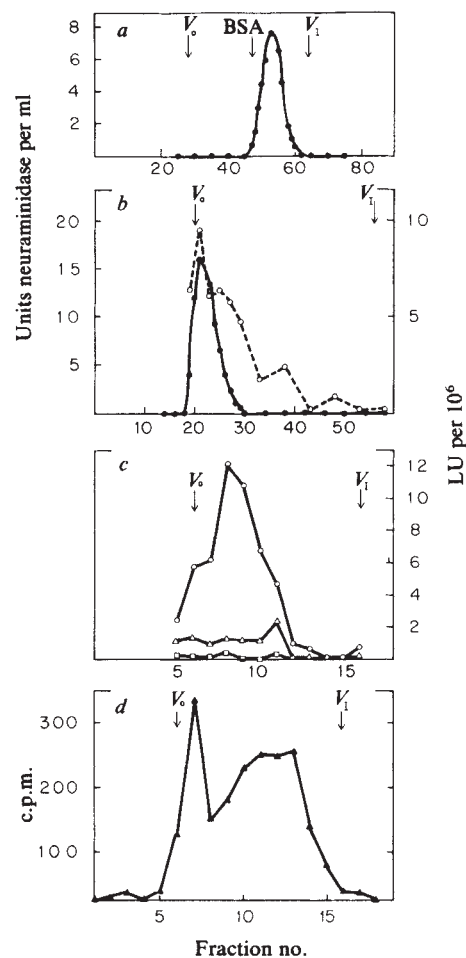


Fig. 4 Characterisation of subtilisin and CNBr-cleaved fragments of Sendai viral proteins. Purified Sendai virus was digested for 60 min at 37 °C with 100 µg subtilisin BPN' (Sigma) (100 µg per 30 mg viral protein per ml) in 0.005 M sodium phosphate buffer, pH 7.4. After digestion, the sample was centrifuged at 200,000g for 60 min, and the supernatant solution containing approximately 10% of the neuraminidase activity in the original virus preparation was chromatographed on a Biogel A-5 m (BioRad, 11 mm × 110 cm) gel filtration column packed with 6 mm diameter glass beads (*a*) and on a Sephadex G-75 (Pharmacia, 21 mm × 55 cm) gel filtration column (*b*) using 0.005 M sodium phosphate buffer, pH 7.2. The subtilisin-solubilised material was also mixed with one-half volume of formic acid and CNBr equivalent to 100 times the estimated mass of the solubilised protein. After overnight cleavage at room temperature, the sample was lyophilised, resuspended in 0.2 M acetic acid chromatographed on a Sephadex G-25 (Pharmacia, 6 mm × 25 cm) gel filtration column in 0.2 M acetic acid (*c*). A separate virus sample (*d*) was subtilisin- and CNBr-digested and then chromatographed on a G-25 column as in *c* except that the starting virus was labelled radioactively using ³H-D,L-alanine injected into the allantoic cavity of the egg used for viral growth. The void (*V*₀) and included (*V*_i) volumes of the column are indicated for each chromatogram. The samples from the columns were analysed for neuraminidase activity (○), radioactivity (▲), and the ability to stimulate CTL. The column fractions were lyophilised, resuspended in TD buffer, and added directly to the cultures for the *in vitro* stimulation as described for Fig. 2. The assays were also as in Fig. 2 except that the target cells included P815(H-2^d) non-infected cells (△), and EL4(H-2^b) Sendai-infected cells (□) in addition to the P815 Sendai-infected cells (○).

Thus it was conceivable that fragments of the HN too small to have neuraminidase activity could still stimulate CTL. After subtilisin-solubilised viral proteins were further cleaved with CNBr, over 99% of the neuraminidase activity was abolished. The cleavage products from radioactively labelled virus were found to have molecular weights ranging from 1,000 (the inclusion limit of Sephadex G-25) to over 5,000 (the exclusion limit of the same gel filtration beads) (Fig. 4d). Fractions from the G-25 column corresponding to proteins smaller than the excluded volume were still able to stimulate BALB/c anti-Sendai CTL (Fig. 4c). Furthermore, these CTL were Sendai-specific and H-2-restricted, lysing H-2^d but not H-2^b cells only after Sendai infection (Fig. 4c).

The material from fractions 19–30 from the column of Fig. 4b was also pooled, cleaved with CNBr and chromatographed on the G-25 column used in Fig. 4c. The same pattern of stimulatory material was observed indicating that compounds of small molecular weight could indeed be generated by CNBr cleavage of larger molecules and retain the stimulatory capacity. When the starting material for the CNBr cleavage from the pooled fractions was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis as described in Fig. 1a, only two bands were seen at molecular weights of approximately 55,000 and 61,000. Because of their large sizes, neither could have been derived from the F glycoprotein. Furthermore, protein corresponding to the smallest detergent-solubilised F peptide (F₁)¹⁰ at molecular weight 51,000 or less was not seen suggesting that no material from the F protein was represented in these G-75 column fractions. However, at least one of the new bands was likely to have been derived from the larger HN by proteolytic cleavage, since neuraminidase activity was still present and the band corresponding to detergent-solubilised HN at molecular weight 69,000 was missing.

In a comparison experiment, the maximum stimulations by the subtilisin-solubilised viral peptides for Sendai-infected P815 target cells, non-infected P815 targets, and Sendai-infected EL4 targets from five independent samples in the plateau region of saturating antigen were 8.4 ± 1.9 , 2.4 ± 0.5 and 0.7 ± 0.2 LU per 10^6 cells, respectively. The corresponding values for the CNBr cleaved material were 5.6 ± 1.7 , 1.6 ± 0.3 and 0.6 ± 0.2 LU per 10^6 cells, respectively, again for five samples in the plateau region. Antigen from fraction 9 of Fig. 4c of molecular weight less than 5,000 was also able to stimulate with corresponding values of 7.3 ± 0.8 , 1.4 ± 0.2 and 0.4 ± 0.1 LU per 10^6 cells, respectively. In all cases, the maximum stimulation was similar to that for the detergent-solubilised HN that is, in the range of 5–10% of the stimulation found for UV-inactivated Sendai virus. Within the limits of sample loss, the CTL stimulation was as efficient using CNBr fragments as it was with the subtilisin-cleaved HN. Therefore, it does not seem that any of the antigenic determinants for this low level of stimulation were destroyed by the subtilisin and CNBr treatments. It is possible that this low level of stimulation corresponds to that found by Finberg, Mescher and Burakoff² using liposomes containing viral proteins in the absence of any H-2 products and probably derives from a special subset of all anti-Sendai CTL precursors. The ability to use isolated viral proteins to stimulate anti-viral CTL has also been reported for influenza virus^{14,15}.

The advantage of using bead-bound antigens stems from the fact that the beads are large and can be removed easily and quantitatively from the *in vitro* stimulation mixture so that the time course of the stimulation can be studied precisely. In initial experiments, the beads lost all their ability to stimulate fresh cells after overnight incubation with responder cells. Therefore, it is conceivable that the antigen bound to the beads was cleaved from them during the incubation and that antigen fragments constituted the actual stimulatory material. Alternatively, the bead-associated antigen could have been processed for the stimulation in some other way. Also, a variety of cells, including macrophages, can be exposed to the beads to determine which cells process the added antigen first. The use of protein fragments in the stimulation tests should permit the biochemical

characterisation of the minimum antigenic determinants for CTL stimulation. As a working hypothesis, we are assuming that one or more cleavage products of HN are responsible for the stimulation since stimulation by the purified detergent-solubilised proteins correlated most closely with the presence of this protein, and, also, analysis of the G-75 column made it unlikely that the F protein was present in fractions which gave rise to stimulatory fragments.

We thank Dr R. Zinkernagel for the hospitality of his laboratory during initial stages of this work and Mrs O. M. Hurlburt for technical assistance. These studies were supported by a PHS research grant.

Received 12 March; accepted 9 November 1979.

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Viral pathogenesis and resistance to defective interfering particles

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Recently presented evidence indicates that synthesis of defective interfering (DI) particles following infection of cultured cells with lymphocytic choriomeningitis (LCM) virus may result in self-curing¹. The genesis of DI particles can occur in cells infected with virtually any animal virus², and seems to be a host-controlled function³. Thus, these particles may represent a third line of defence—with the immune response and interferon—against virus invasion^{3,4}. LCM virus infection causes the rapid appearance of DI particles in tissue culture cells^{5,6}. Nevertheless, persistent infections can be easily established with LCM and other arenaviruses^{7–9}. The LCM-persistent infections are apparently maintained in these cultures by the genesis of DI-resistant viruses¹. This latter finding prompted us to determine whether such DI-resistant viruses occur in nature. Vertical transmission of LCM virus in the house mouse or neonatal infection of laboratory mice takes the form of a life-long inapparent infection. These mice lack functional virus-specific T lymphocytes, but may make an antibody response against the invasion. It is known that LCM virus injected into a neonatal mouse can change during the course of the infection so that at least two different viral populations coexist in the same mouse¹⁰. DI particles are produced in abundance shortly after neonatal infection and this precedes the appearance of a virus population that, by plaque morphology, differs from the one originally inoculated¹¹. We report here the isolation of an LCM virus from persistently infected mice that is resistant not only to the DI particles which were produced shortly after neonatal infection with heterotypic virus but also to the DI particles that this virus itself induces in neonates. We know of no other report of viruses which can replicate normally in the presence of the DI particles they induce.

A recently isolated clone of the UBC strain of LCM, designated substrain T, was injected into outbred (ICR) mice less than 24 h old. This virus strain had a long history of tissue culture passage in our laboratory¹². Ten months later, the blood of these mice exclusively contained two virus types which were easily distinguishable from substrain T (Table 1). These cloned viruses were called substrains A and D because the disease syndromes they produced in mice infected as adults were similar (Table 1) to two previously described substrains of LCM-UBC designated 'aggressive' and 'docile' (ref. 13). All three virus types: (1) were neutralised by heat-inactivated (56°C for 30 min) filtered ascites fluid taken from a patient infected with the MP strain of LCM; (2) induced patching and capping on living MDCK cell membranes after a fluorescein isothiocyanate indirect staining sequence using sera from a substrain T hyperimmunised hamster; (3) initiated life-long (>9 months) persistent infections when injected into neonatal ICR mice. DI particles for each of the three substrains, designated DI_T, DI_A and DI_D, were generated in neonatal mice by previously described methods¹¹. Each newborn mouse in a litter (usually 15) was injected intraperitoneally with 100 LD₅₀ of one of the substrains. Eight days later the mice were killed and kidneys from the littermates were pooled and homogenised. After resuspension in 10 ml of tissue culture medium and clarification by low-speed centrifugation, the putative DI particle stocks were stored at -70°C until use. DI particle activity was measured in MDCK cells. These cells are killed with single hit kinetics by infection with standard virus. However, co-infection with a single DI particle not only spares the cell but also inhibits the replication of standard virus¹⁴. Our test was to infect a series of MDCK monolayers with one type of DI particle and then challenge them individually with each of the three virus substrains shown in Table 1 (which by themselves would cause almost complete destruction of the monolayer). DI particle activity was determined visually and by reduction of the amount of infectious challenge virus synthesised.

In each case, all three putative DI particle preparations completely inhibited any observable cytopathic effect (CPE) caused by standard virus substrains T and D (Table 2). Synthesis of these challenge standard viruses in the above conditions varied from 13% to 43% of normal (we have previously shown¹⁴ that suppression of CPE and inhibition of standard virus synthesis by DI_T particles are separable functions). However, none of the DI preparations protected cultures challenged with substrain A (Table 2). Thus, substrain A seems to be relatively resistant to DI particles in general, even to those that it induced.

The results presented in Table 2 were judged to be due to DI particles for the following reasons. (1) Kidneys taken from normal mice had no sparing ability on cultures challenged with any of the three standard viruses. (2) UV treatment of the DI particle-containing kidney extracts in conditions which lowered

Table 2 Ability of DI particles to interfere with the synthesis of different standard LCM viruses

Standard challenge virus subtype	% Of control challenge virus yield after pretreatment of MDCK cells with:			
	DI _T *	DI _T	DI _A	DI _D
A†	95	90	94	94
D‡	13	32	23	26
T‡	25	30	43	37

Kidney extracts, which were found to contain the highest amount of DI particles after neonatal infection¹¹, were diluted so that 3 ml of tissue culture fluid contained the equivalent amount of virus extractable from one organ. Aliquots of 1 ml of the virus were then used to infect a 25-cm² monolayer of MDCK cells as described previously¹⁴, or first subjected to the UV irradiation from two 30-W GE UV lamps giving an incident energy dose at the surface of the fluid of 6×10^4 erg. After a 1.5-h adsorption period the cells were repeatedly washed and then challenged with standard virus at an MOI of 0.1. The cultures were observed under the microscope at intervals over a 72-h period. DI particle-treated cultures were always compared with those that had either no pretreatment or pretreatment with organ extracts from normal mice. All tissue culture fluids were changed at 40 h post-infection and saved for future plaque assay titration. Control challenge virus yields were: substrain A, 1.3×10^8 plaque forming units (PFU) per ml; substrain D, 4.6×10^8 PFU per ml; substrain T, 3.0×10^8 PFU per ml.

* This preparation was not UV irradiated.

† All DI particle-pretreated cultures challenged with substrain A developed cytopathology to the same extent as cultures infected with substrain A alone.

‡ All DI particle-pretreated cultures challenged with substrains D or T developed little, if any, cytopathology.

the infectivity of standard virus 100-fold (multiplicity of infection (MOI) reduced from 0.7 to 0.007) had little or no effect on the cell-sparing phenomenon or the inability of these cells to support the replication of challenge virus (Table 2). This agrees with the previous observations of the relative UV resistance of LCM DI particles^{15,16}. Furthermore, UV treatment did not increase the interference phenomenon, thus eliminating the possibility that it was an artefact created by irradiation of standard virus. (3) LCM-specific antiserum treatment of the kidney extracts taken from mice infected with substrain T lowered the standard virus titres (and supposedly the DI particle titres, as its polypeptide composition is not recognisably different from that of standard virus¹⁷) by 90% and effectively eliminated the cell-sparing and virus inhibitory properties. These results, combined with the unique properties of the DI_A particles, eliminated interferon as an explanation of the results. (4) Organ extract addition immediately after a 1.5-h adsorption period with 'challenge' virus still exerted its inhibitory effect on the virus, albeit somewhat less effectively¹⁴. This eliminated the possibility that the observed effects were due to blocking of receptor sites for the challenge virus or neutralising antibody in the kidney extracts. All these results are consistent with the general properties of DI particles². When it becomes possible to separate LCM DI particles from standard virus effectively by physical means, purification of the DI particles from the kidney extracts can be undertaken.

Tissue culture models^{1,5} of LCM persistent infections continue to have predictive value when applied to *in vivo* situations. The present finding of a DI-resistant virus offers an explanation for one of the plaque morphology changes in the virus as life-long infection progresses. The virus-cell interactions in these mice are obviously complex. The role of LCM substrain D is unclear; the time course of its appearance with regard to substrain A is under investigation. An LCM virus which changes slightly, if at all, in plaque morphology has also been observed in persistently infected mice¹⁸. This virus may initially have been resistant to DI particles, or evolved to this state without other striking signs of change. Our studies suggest that it may be worthwhile to determine whether DI-resistant viruses have arisen through natural selection in other persistent

Table 1 Plaque morphology and mortality pattern as a function of LCM virus substrain

Virus designation	MDCK plaque type* (diameter)	Murine characteristics†		
		Appearance at death	% Dead	Mean day of death ± s.d. (range)
T	Clear (3–4 mm)	Convulsive	100	9.7 ± 1.4 (7–11)
A	Pinpoint (1–2 mm)	Convulsive	100	6.75 ± 0.5 (6–7)
D	Clear (3–4 mm)	Wasting	75	12.3 ± 5.8 (9–19)

* Monolayers were stained with neutral red on the fourth day after infection and plaques were scored within 24 h.

† Groups of 12 ICR mice, 14–21 days old, were injected intracerebrally with 100 LD₅₀ of virus stock in a volume of 0.02 ml. Although a clear LD₅₀ end point was obtained with substrain D, it was routinely observed that mortality fell as the dose of the inoculum increased.

infections, especially those in which mutant viruses have appeared.

It has been proposed that cells budding LCM virus in the choroid plexus, ependyma and meninges are specifically recognised and damaged by sensitised T cells^{19,20}. As DI particles suppress surface antigen expression in LCM-infected cells^{21,22}, the pathogenetic mechanisms of various LCM viruses in the adult mouse may be linked to their resistance to DI particles.

Received 7 August; accepted 13 November 1979.

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¹H NMR study of valinomycin conformation in a phospholipid bilayer

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Our understanding of how ions pass across biological membranes has been greatly advanced by the study of small molecules which are capable of enhancing ion transport. The concepts of ion movement through channels or via mobile ion carriers have arisen from studies of model systems^{1–4}. However, direct probing at the molecular level of the process of ion movement in a membrane system has proved difficult. The electrical properties of black lipid membrane model systems do not provide information about the details of ionophore location or conformation. Spectroscopic methods which are suited for probing the details of ionophore conformation and the stoichiometry of ion binding have been confined largely to organic solvent systems which are limited as models for biological membranes. We report here proton magnetic resonance (¹H NMR) spectroscopic studies which investigate valinomycin conformation and ion binding in small bilayer vesicles.

Valinomycin is a cyclic 12-membered depsipeptide composed of D-valine, L-lactic acid, L-valine and D-hydroxyisovaleric acid with the structure [D-Val-L-Lac-L-Val-D-HyIv]₃. It is an interesting ionophore because of its ion binding selectivity. It is particularly suitable for the work reported here because its conformational states are now well understood, largely owing to extensive NMR^{5–8}, circular dichroism^{6,9}, and infrared^{6,9} studies in solvents, X-ray diffraction studies of crystals^{10,11}, and conformational energy calculations^{12,13}. The present studies in phospholipid bilayers were made possible by our recent synthesis of a perdeuterated phospholipid, dimyristoyl phosphatidylcholine (DMPC-d₇₂) (ref. 14). DMPC-d₇₂ has 99.0–

99.7% replacement of ¹H by ²H throughout the molecule. The corresponding reduction in the ¹H NMR background signal makes it possible to observe ¹H NMR spectra from small molecules which are incorporated into bilayer vesicles of the perdeuterated lipid¹⁵.

DMPC-d₇₂ was synthesised according to Kingsley and Feigenson¹⁴. Valinomycin was dissolved in chloroform together with the phospholipid, and solvent removed under high vacuum. Small bilayer vesicles were prepared by sonication in ²H₂O, or in a NaSCN solution in ²H₂O having ionic activity of 2.25 M. Alkali cation titrations were performed by adding the thiocyanate salts to the vesicle samples. The exclusive location of the valinomycin within the bilayer and not in the water was determined by chromatography with Sepharose 4B. The valinomycin profile followed the phospholipid profile in both 50 mM NaHPO₄ buffer, pH 7, and in 3 M KSCN.

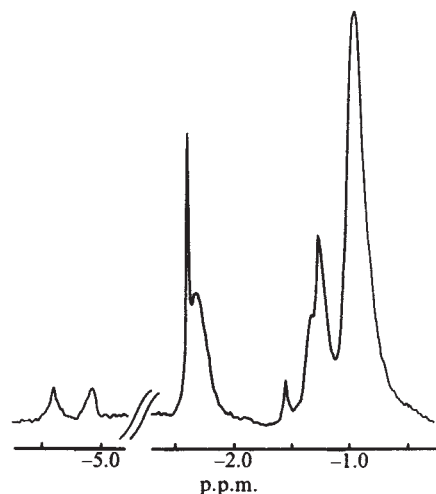


Fig. 1 360-MHz proton magnetic resonance spectrum of 3.1 mol % valinomycin in 11 mg ml⁻¹ small sonicated bilayer vesicles of DMPC-d₇₂ with 50 mM NaHPO₄, pH 7, at 50 °C. Transients (48) were collected and a sensitivity enhancement corresponding to a 5-Hz linebroadening was applied. The peak assignments are as follows: -5.39 p.p.m., L-Lac C_αH; -5.07 p.p.m., D-HyIv C_αH; -2.38 p.p.m., CH₃ from trace tosylate impurity; -2.32 p.p.m., C_βH from valinomycin plus residual lipid acyl C₂HH; -1.55 p.p.m., residual lipid acyl C₂HH; -1.33 p.p.m., L-Lac CH₃; -1.27 p.p.m., residual lipid acyl (C²HH)_n; -0.93 p.p.m., D-HyIv and D,L-Val CH₃.

Resonances from L-Lac and D-HyIv C_αH and CH₃, from D, L-Val CH₃ and from D, L-Val and D-HyIv C_βH groups are well-resolved at 360 MHz for valinomycin incorporated into DMPC-d₇₂ vesicles in the absence of alkali cations (Fig. 1). Assignments are according to Ohnishi and Urry⁵. Chemical shifts are given in Table 1. The linewidths of 20–40 Hz for the valinomycin resonances, similar to the linewidths for the phospholipids or for other small molecules incorporated into small bilayer vesicles¹⁵, imply considerable local molecular motion. The chemical shift values observed and the temperature dependence of these shifts are close to the values observed in non-polar organic solvents (see Table 1 and refs 8, 16). Spin-spin splitting could not be measured because of the linewidths of 20–40 Hz. This is unfortunate, as the Karplus-type relationship between the coupling constants and the molecular conformation is well-established for many peptides and for valinomycin in particular⁸. Instead, we used the empirical relationship between the observed chemical shifts for valinomycin and its molecular conformation^{7,8,16}. Thus, the conformation of uncomplexed valinomycin in bilayer vesicles is the 'A₂ bracelet', with quasi-axial carbonyl groups, previously described for valinomycin in cyclohexane and in chloroform⁸. Because of this assignment of the conformation of uncomplexed valinomycin, it is reasonable

Table 1 Proton chemical shifts for valinomycin and its alkali cation complex at 50 °C

Proton	Residue	Chemical shift				
		Vesicles, uncomplexed	Vesicles, Rb ⁺ complex*	Cyclohexane, uncomplexed	Dimethylsulphoxide, uncomplexed	Chloroform, K ⁺ complex†
C _α H	L-Lac	5.34	5.04	5.31	5.09	4.90
	D-HyIv	5.04	4.61	5.03	4.82	4.55
	D-Val	4.16		4.03	4.26	3.74
	L-Val	4.03	3.72	3.91	4.36	3.85
C _β H	D, L-Val, D-HyIv	2.32	2.22	2.34	2.18	2.16
CH ₃	L-Lac	1.36	1.55	1.36	1.28	1.51
CH ₃	D, L-Val, D-HyIv	0.96	0.96	1.00	0.91	1.02

* Extrapolated values.

† Data from ref. 8.

to infer that the location of uncomplexed valinomycin is in a largely non-polar environment. Support for the inference that the predominant location of uncomplexed valinomycin is in the hydrocarbon region of the bilayer must await further investigation.

The chemical shifts of the L-Lac, L-Val, and D-HyIv C_αH and the L-Lac CH₃ can be followed up to high concentrations of added alkali salts, thereby describing a complete ion binding isotherm. The ion binding curves for Rb⁺ are shown in Fig. 2. Similar results were obtained for K⁺. Addition of up to 3M NaSCN caused no significant chemical shifts, in accordance with the extremely weak binding by valinomycin for this ion in aqueous systems^{9,17}. The values for the limiting chemical shifts of L-Lac, L-Val, and D-HyIv C_αH and of L-Lac CH₃ resonances at high concentrations of K⁺ or Rb⁺ are very similar to the shifts observed in a variety of organic solvents for the fully complexed ionophore⁸. Thus we conclude that the solvent and the bilayer conformations of the fully alkali cation-complexed valinomycin are both of the 'bracelet' type, with carbonyl groups directed inward.

During the course of these titrations each resonance remains as a single peak, up to a shift of as much as 150 Hz for the D-HyIv C_αH. Thus, complexed and uncomplexed valinomycin are in fast exchange on this time scale. Any steps in the mechanism of K⁺ or Rb⁺ binding or release by valinomycin must be significantly faster than 150 s⁻¹. Fast exchange for cation complexation has previously been measured for valinomycin in black lipid membranes¹⁸. This rate must apply to the transfer of the monovalent cations between water and valinomycin, a necessary step in the association. If the ion binding mechanism should also involve a change in the location of the uncomplexed

valinomycin, for example from a position in the bilayer interior to a position near the aqueous interface, then this process too must be fast on this time scale. Note that for valinomycin in organic solvents the exchange rate between complexed and uncomplexed forms is slow on this time scale¹⁹.

The titration curves shown in Fig. 2 are hyperbolic, as would be expected for 1/1 binding. Thus this experiment shows no evidence of the formation of complexes of other stoichiometries²⁰. When titration of valinomycin is performed by simply adding Rb⁺ or K⁺ salts to the vesicle suspension with no added NaSCN, thus allowing the cation activity to vary from 0.03 to 0.60 M, the observed chemical shifts were significantly smaller over the first half of the titration than are the shifts shown in Fig. 2. Therefore, any positive charge contributed to the vesicle surface by cation-valinomycin complexes must be more than offset by the adsorption of lipophilic thiocyanate anions in the experiments shown²¹.

In conclusion, we have presented the first ¹HNMR studies of the conformations of valinomycin within a phospholipid bilayer. We find that uncomplexed valinomycin has a similar conformation in the bilayer and in non-polar organic solvents. Thus, the uncomplexed valinomycin molecule could be located predominantly in the interior of the bilayer. This conformation shifts on K⁺ or Rb⁺ binding to a structure which is characteristic of fully complexed valinomycin in many different solvents. Complexed and uncomplexed valinomycin are in fast exchange (rate > 150 s⁻¹) throughout the observed range of alkali cation activities of 0.5–600 mM. Further work with this system is now directed toward determining the location and the dynamics of valinomycin in the phospholipid bilayer.

This research was supported by NIH grants HL 18255 and GM 25675. P.R.M. received support from NIH Research Service Award 5 T32 GM07273. This work was carried out, in part, at Brookhaven National Laboratory under the auspices of the United States Department of Energy.

Received 31 May; accepted 9 November 1979.

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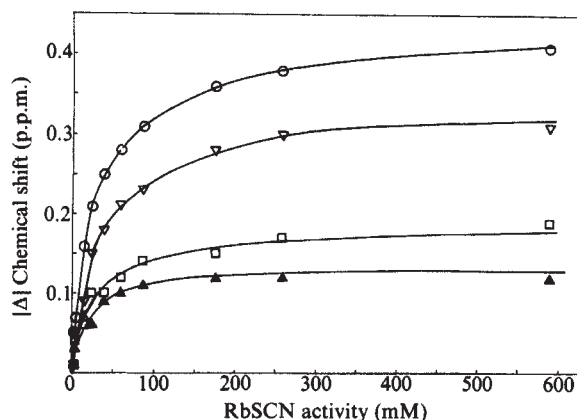


Fig. 2 Rb⁺ binding curves for 3.1 mol % valinomycin in DMPC-d₇₂ vesicles at 50 °C. The change in chemical shift is plotted against the activity of RbSCN. The activity coefficients used were those for KSCN (ref. 22). ○, D-HyIv C_αH; ▽, L-Val C_αH; □, L-Lac C_αH; ▲, L-Lac CH₃. The sample contained the appropriate concentrations of NaSCN to keep the ionic activity constant throughout the titration.

BOOK REVIEWS

Elementary particle theory

R.H. Dalitz

EVEN a decade ago, it was apparent that the proton and neutron, previously regarded as elementary constituents of the atomic nuclei, were actually composite. Very many excited states were known for them and many also for the hyperons and the mesons. The quantum numbers of these excited hadronic states formed definite patterns, recognised to correspond to a 'molecular model' for the hadrons, each baryon consisting of three of the quarks hypothesised by Gell-Mann and Zweig some years before, each meson consisting of a quark and an antiquark. Each pattern corresponded to a definite excitation of their internal spin and orbital motions. Independently, the data on deep-inelastic electron-nucleon scattering had shown rather directly that nucleons contained point-like particles, which Feynman termed 'partons'. With later experiments on deep-inelastic $\nu \rightarrow \mu^-$ and $\bar{\nu} \rightarrow \mu^+$ reactions on nucleons, these data showed that partons and quarks appeared to be identical, having the same spin, baryon number and charge values ($\frac{1}{3}$ and $-\frac{1}{3}$ times the proton charge). This quark-parton picture of the hadrons is now generally accepted.

The quarks are 'point-like' for distances down to 10^{-16} cm, at least, so that they are our present-day candidates for the title 'elementary particles'. However, quarks come in a number of kinds, with differing attributes, known as 'flavours'. There are $N=5$ flavours established today, the corresponding quarks being labelled u, d, s, c and b, in order of mass, and the discovery of further flavours is expected. The experiments also showed that nucleons contain neutral objects; these are now believed to be the quanta of a 'gluon field' which binds the quarks and antiquarks into the hadron. Finally, in order to give the patterns observed, the Pauli principle required that each quark have three possible states, each now labelled by a colour, and each indistinguishable through the hadronic, electromagnetic and weak interactions we know. This corresponds to an exact 'colour symmetry' and we now believe that the gluon field may be the gauge field for 'colour', just as the photon field is the gauge field for charge. This gauge theory is now known as quantum chromodynamics (QCD). It is a tightly constrained theory, almost unique in form, and it is the theory at the centre of attention today.

Following QCD, elementary particle theory is now undergoing rapid changes and our views today differ greatly from

An Introduction to Quarks and Partons. By F.E. Close. Pp.481. (Academic: London, New York and San Francisco, 1979.) £26.40; \$54.50.

those when this book was finalised. Nevertheless, the book will be much welcomed, especially by students, for it is the only recent introduction available at book length. It does not proceed to expound the subject deductively, from basic principles, but is better described as a discursive review surveying the state of the art in this field of physics in mid-1978. It tends to give results without derivation, although then analysing the result to give some intuitive understanding of its origin and meaning, a procedure having great value for any isolated reader.

Following a brief introduction to set the scene, the book consists of three parts. The first part begins with $SU(N)$ symmetries for N quark flavours, initially for $N=2$ and then for $N=3$. This $SU(3)$ symmetry is then extended to an $SU(6)$ symmetry in the space $SU(3) \times SU(2)_\delta$, where δ denotes Pauli spin. In this framework the quark model classifies hadrons according to the product group $SU(6) \times O(3)$. Since Pauli spin is non-relativistic, this classification cannot refer to a fundamental symmetry; its use today is motivated only by the quark model and is for convenience, allowing the systematic procedures of group theory to be used for economic calculation and tabulation. In general, the observed hadrons correspond to mixed representations of $SU(6) \times O(3)$. The matrix-elements, for the most important transitions, are shown to correspond to the group $SU(6)_W$, so its algebra is discussed in considerable detail, making the usual distinction between $SU(6)$ for currents and $SU(6)$ for hadron constituents and leading to the general Melosh expression for electromagnetic transition amplitudes. Various selection rules are noted, compared with the data and analysed to show how they work. For hadronic decay processes, the transition 'Vacuum $\rightarrow {}^3P_0(\bar{q}q)$ ' is introduced, and the resulting amplitudes are related with $SU(6)_W$. Some review is given of attempts to include relativistic effects in quark-model calculations.

The second part is devoted to partons and their phenomenology. The notion of levels of scaling is introduced, and traced (for inelastic electron scattering) from the nuclear to the quark-parton level. The parton distributions in nucleons are deduced from the various leptonic deep-inelastic reaction data and discussed in

considerable detail, bringing out their connection with simple field theories. There are brief discussions of scale invariance in electron-positron annihilation at high energies, of neutral currents appropriate to the unified electromagnetic-weak interaction, and of the observed scaling violations, now believed to reflect asymptotic freedom for the underlying field theory. The parton model is extended by introducing quark fragmentation functions, used to analyse and predict hadron spectra and their angular dependence in various high-energy reactions. This leads on to a discussion of large momentum-transfer phenomena in hadron-hadron collisions and to quark counting rules for their asymptotic forms. Specific models such as 'Drell-Yan' and 'constituent-interchange', are introduced and compared with the data.

The third part first sketches briefly the electromagnetic-weak and QCD gauge theories, analysing the qualitative features of the one-gluon-exchange potential between quarks. The major topic is the charmed quark c and its role in the 'new particles', such as the ψ/J and ψ' mesons discovered in 1974, and the D and F mesons established subsequently. Quite a detailed discussion is given of the bound states of Charmonium ($c\bar{c}$), the 'hydrogen atom' of the 'new particle' physics; all of its properties are well accounted for by QCD, assuming that this can be shown to imply confinement. The heaviness of the c quark (about 1.5 proton masses) justifies a non-relativistic Schrodinger treatment and the hyperfine structure of the spectrum arises from the one-gluon-exchange potential. Electric dipole γ -transitions occur between its S and P states with rates in accord with these Schrodinger calculations. The long lifetimes of the ψ/J and ψ' states are due to the asymptotic freedom of QCD. The one-gluon-exchange potential and the quark masses are then used to account for all of the mass splittings between and within the various charge multiplets observed within particular $SU(6) \times O(3)$ mesonic and baryonic supermultiplets. There are also chapters dealing with the 'bag model' for hadrons and the possibility of bound multi-quark systems. The former is entitled "Quarks confined to a sphere"; despite its very general formulation, the bag model program has been implemented to date only for the case with a rigid spherical bag. The book closes with gauge theories for 'grand unification', where quarks and leptons are assigned to common multiplets and the lepto-quark gauge fields are

extraordinarily heavy, with masses of the order of $10^{-10}g$. These theories mostly imply that the proton should decay spontaneously (for example, to $e^+ + \pi^0$) and have led to a number of experimental proposals to push up the present limit ($\lambda_p \sim 10^{30}$ years) on the proton lifetime.

This is a book for the quark-model practitioner. It gives all the prescriptions and rules; they are analysed thoroughly but are not always derived (nor even necessarily all consistent). It says something about every idea current today, although some in much more detail than others. The book is somewhat uneven in its treatment, being strongest and most detailed in the areas where the author's own contributions lie (photo-excitation of excited baryons and polarised lepton-hadron interactions). It also has a somewhat dated air, since our picture of elementary particle physics has been changing rapidly. It is now a general belief that the quarks (u,d) are almost massless, whereas most hadron spectroscopy still assumes non-relativistic motion for the quarks in hadrons. The $SU(N)$ symmetries discussed here are now considered accidental, the true symmetry being the flavour independence of quark-gluon

interactions, violated only by the quark mass values. Little is said about 'confinement', the central unresolved question for QCD; indeed, the book says rather little about QCD, beyond implications of the one-gluon-exchange potentials. The book has come too soon to include the recent developments in baryon spectroscopy due to Isgur and Karl, which have brought much order concerning Λ^* and Σ^* states. It would have greater value for students today if it had more about the fundamentals and applications of gauge theories, especially for QCD, since the book would then have appeared forward-looking; its present emphasis is on theoretical aspects from the past, which may be expected to change in the near future. But this is perhaps the fate of any book in this field of physics, where exploration is moving fast; it is easy and unfair to make this judgement in retrospect. There is no doubt that every physics library should possess this book, for there is much to be found on its pages which it is not easy to find elsewhere. □

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Chondrocytes and their matrix

F.N. Ghadially

Biology of Cartilage Cells. By R.A. Stockwell. Pp.329. (Cambridge University Press: London, New York and Melbourne, 1979.) £25.

THIS book is a welcome addition to the literature on cartilage. It is not a book about the chondrocyte only, as the title might lead one to believe; quite detailed accounts of the morphology and chemistry of various components of the matrix are included as well as other topics such as the nutrition and permeability of cartilage and the nature of the articular surface. All this is successfully accomplished in what the author modestly refers to as a "short book", but the concise style of writing has permitted the inclusion of a volume of factual data which one usually associates with much larger works. The well selected references and a carefully prepared index enhance the value of this work.

It is difficult to fault the text in any major respect. The photomicrographs are rather small in size but of good quality; the same, however, cannot be said of the electron micrographs, which are of a variable quality. In contrast to this the line drawings are superb, and the graphs and tables are well planned and informative.

The book begins (chapter 1) with a brief description of the morphological and chemical differences between various types of cartilage. Chapter 2 gives an adequate account of the ultrastructural morphology of the chondrocyte, the effect of hormones on the chondrocyte and the antigenicity of chondrocytes. Chapter 3 deals with the morphology and chemistry of the matrix components. In this excellent chapter we are treated to quite a detailed account of collagen structure and synthesis, elastic fibres and elastogenesis, and the structure of proteoglycans. However, the morphology of proteoglycans (that is, matrix particles) is barely discernible in the electron micrographs presented. Chapters 4 and 5 deal with the metabolism and nutrition of chondrocytes. Chapters 6 and 7 deal with chondrocyte differentiation and proliferation. The book ends (chapter 8) with a discourse on degenerative changes, age — associated changes, and a section on calcification in which an up-to-date account of matrix vesicles and their role in cartilage calcification is presented.

In summary, this book provides a brief review of existing knowledge about the chondrocyte and surrounding matrix. It is up to date, well written and packed with factual data. In my opinion this book should be of value not only to research workers as suggested by the author, but also to rheumatologists, orthopaedic surgeons and postgraduate students (residents) interested in cartilage.

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Primary energy resources

D.O. Hall

Biological Energy Resources. By M. Slessor and C. Lewis. Pp.192. (E. & F. N.Spon: London, 1979.) £8.50.

TODAY about one-seventh (or possibly more) of the world's primary energy is derived from biomass — this is equivalent to 19 million barrels of oil a day (which is twice the Saudi Arabian oil production and equal to the US daily oil use). However, because most of this biomass energy use occurs in the developing countries (and there predominantly in rural areas) and because it seldom enters the official statistics, it has, until recently, been virtually ignored by planners, politicians and aid agencies alike. A combination of the "energy crisis" and desertification problems has focussed the attention of both developing and developed countries on the importance and potential of 'biological energy resources'.

This aptly titled book is a must for anyone interested in the basics of bioenergy and its conversion, and more especially in the energetics and economics of biomass systems. The concepts of energy ratios, net energy yield, net utilisable energy production, and gross energy requirement are well presented, and a number of case studies are thoroughly discussed. It is clearly pointed out that intensification of natural ecosystems for biomass energy requires both capital inputs and better management — but local industry, skills and energy self-reliance can be built up. Such energy systems are applicable to varying extents in diverse conditions around the world and must be tailored to suit local conditions.

The chapter on economics of biomass systems touches on net present value, energy payback times, and economic costings. It considers some specific examples like ethanol production and biogas generation, and compares costs with other energy sources. These costs are changing so rapidly it seems imperative to update one's analyses and comparisons annually to take account of accelerating costs.

It is a good book and very timely. But please could authors and/or publishers try to list references that can be traced — conference proceedings references are useless unless the accurate source is given and organizations who publish booklets, and so on, have their addresses given. In such a rapidly developing field with wide sources of interest it is important to be able to trace the information. □

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Grassland ecology

Peter D. Moore

Grassland Ecosystems of the World. (International Biological Programme, 18.) Edited by R.T. Coupland. Pp. 401. (Cambridge University Press: Cambridge, 1979.) £25. *Perspectives in Grassland Ecology: Results and Applications of the US/IBP Grassland Biome Study.* Edited by Norman R. French. Pp. 204. (Springer: New York, Heidelberg and Berlin, 1979.) DM54; \$29.70.

SOME thirteen years after the initiation of the International Biological Programme for the collection of information concerning the productivity of the world's biomes, the publication of the accumulated data is gaining momentum. The book books reviewed here are concerned with grassland ecosystems; the first (edited by Coupland) is a condensed comparative synthesis of information from various sites around the world, and the second (edited by French) is a more detailed set of papers from the United States IBP grassland team.

In some areas of the world grassland is believed to represent the climax vegetation type, existing as a response to climate, soil, natural grazing or fire. In other regions, grasslands have developed as a result of human management practices. Often it is difficult to distinguish the two with certainty. In Coupland's book, grasslands are divided initially into climatically determined types and those actually sown and managed by man. Then, within the climatic subdivision, natural and semi-natural grasslands are distinguished — the latter being exploited by man either for hay crops or for grazing.

Each type is dealt with in a separate section subdivided into chapters concerning primary production, consumer organisms, microorganisms, ecosystem synthesis and modelling, nutrient cycling, and the exploitation, management and conservation of grassland systems. This arrangement is particularly valuable, as it permits an easy cross-reference from one

section to another. It makes it simple, for example, to check back from tabulations of the efficiency of energy capture in Indian grasslands to equivalent data in a similarly constructed table for prairie systems in the United States.

This facility reflects creditably upon those responsible for the design of the volume and upon the editor, who has ensured a uniformity of expression and presentation which greatly aids those who wish to obtain comparative figures with ease. This must be regarded as the primary function of a book like this one, which seeks to present a global collation of IBP grassland data.

Perhaps a surprising inclusion in the book is a section on croplands, reviewing graminaceous agroecosystems. Sadly, this is the least informative section, containing many casual pieces of information in a generally uncoordinated manner. It is largely derived from data emerging from the Polish Agroecological Station at Turew, Poland, but really a wider synthesis of world literature is necessary if this were to be a useful discussion.

Coupland concludes the volume by attempting to make generalisations concerning the structure and production of grasslands. This is a formidable task and tends to take the form of a summary of the range of variation found within grasslands of different types. It is useful mainly in that it identifies those areas of study which need further attention, such as the role of fire in grassland management.

Perspectives in Grassland Ecology, edited by N. French, is more limited in its geographical coverage, being solely concerned with the US grasslands, but it is far more diverse in its structure. It is essentially a series of independent papers concerning grassland studies in North America. Some, such as Lauenroth's paper on grassland primary production, are attempts to review areas of grassland study and to place them in their wider context. Others are concerned with very specific research topics, such as Detling's study of the factors controlling the productivity of the C4 grass *Bouteloua gracilis* in mixed grassland in Colorado.

The collection of papers deals with a range of topics, many of which will be of interest to ecologists in general, such as the role of stress in grassland ecology (Dodd and Lauenroth), biomass trophic pyramid structure in grasslands (French, Steinhart and Swift) and nitrogen input and output (Woodmansee). Finally there is a series of papers concerning ecosystem modelling in grasslands and its value in management studies.

The two books are thus very different in approach. Both supply valuable sources of information and current ideas in the field of grassland ecology. □

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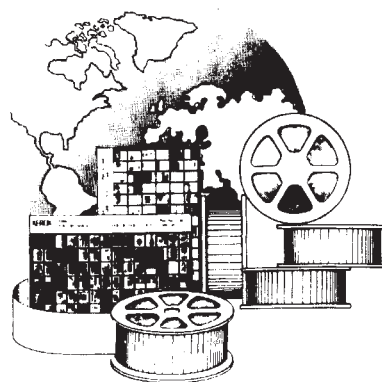
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● *A Handbook of Numerical and Statistical Techniques* by J.H. Pollard (for review, see *Nature*, 270, 284; 1977) has now been published in paperback by Cambridge University Press at £5.50.

● *Problems of Genetics* by William Bateson, first published in 1913, has now been published with an historical introduction by G. Evelyn Hutchinson and Stan Rachootin, by Yale University Press (New Haven and London) at £12.30 hardback and £3.15 paperback.

Particle transport processes

W.A. Wakeham

Transport Theory. By J.J. Duderstadt and W.R. Martin. Pp.613. (Wiley: New York and Chichester, UK, 1979.) £24.

THE study of particle transport processes in a wide variety of fields has been a growing area of activity for a number of years. Inevitably, the developments in the various fields have been largely independent and the underlying similarity of the governing equations for particle transport has been somewhat neglected. This book continues and enhances the modern trend of providing a common basis and methodology for the study of all transport processes and brings together an armoury of techniques for the solution of transport problems.

The fundamental proposition of the book is that transport theory is the mathematical discipline associated with the solution of transport equations. This restricted, but valid, view leads to a volume which is more suited to applied mathematicians than to physicists or engineers. The essential physics of any transport

problem is the derivation of a kinetic equation for the problem, the evaluation of the various scattering cross-sections and a statement of the boundary conditions. Of these, only the derivation of kinetic equations is given significant attention in this book. However, the familiar Boltzmann, Fokker-Planck and Vlasov transport equations are obtained with minimum fuss and a short description of the projection operator methods for the derivation of more general transport equations is included. The relationships between the kinetic equations, continuum theory and non-equilibrium statistical mechanics are given a careful and useful exposition.

The elegant mathematical techniques for the solution of transport equations in the integro-differential or integral form, which make up the essence of the book, are well presented. First the simplest transport equations for idealised non-scattering single energy systems are solved and then several more complicated problems are attacked. The necessary mathematical tools are generally developed as required, but a potential reader should already be well versed in integral transforms, boundary value problems and complex variable analysis before attempting the book. Otherwise the treatment of integral equations will be insufficient and the "Child's Primer on the Spectral Theory of

Operators", provided in an appendix, will remain obscure. The tool kit for the solution of transport problems is completed by a discussion of numerical methods including finite element techniques and the Monte Carlo simulation methods.

The full battery of mathematical tools is unfortunately applied to very few practical examples in a few problems surprisingly included at the end of every chapter. It is rather as if, when training a mechanic, the theory of a screwdriver was carefully explained but he was never required to use the device to undo a screw. As a practical scientist I found this aspect of the book disappointing since the fields to which transport theory can be applied range from the important subject of neutron distributions in nuclear reactors through light propagation in stellar atmospheres to vehicular traffic flow.

However, the book is written with sufficient humour and insight to lift it above the level of a mere compendium of mathematical methods. In any event, no scientist interested in more than a phenomenological description of particle transport should be without this volume.

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Signal processing

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An Introduction to Statistical Signal Processing, with Applications. By M.D. Srinath and P.K. Rajasekaran. Pp.499. (Wiley: New York and Chichester, UK, 1979.) £18.30. *Digital Signal Processing and Control and Estimation Theory: Points of Tangency, Areas of Intersection, and Parallel Directions.* By A.S. Willsky. Pp.256. (MIT Press: Cambridge, Massachusetts, and London, 1979.) \$22.50; £13.50.

THERE are already numerous introductory textbooks on signal processing but the temptation to turn their lecture notes into a textbook continues to be found irresistible by some university lectures. Furthermore, in such a subject, which is expanding into new areas, there is always some fresh application to mention that was not covered in earlier texts. The first of these two volumes is just such a book; it arose from various courses of lectures in the Southern Methodist University (Dallas) and two Indian institutes, and covers detection and estimation theory in communications, radar, pattern recognition

and system identification. It is a competent if uninspired work, clearly written for the most part though rather pedestrian in tone, and contains many worked examples, for which students will be grateful. Although it is described by the authors as a graduate text, much of the material is taught at undergraduate level in British universities, and for this audience, the book is probably too detailed: lecturers and students alike will probably find that their preferred text on signal processing, supplemented by a volume on two-dimensional questions in general (pattern recognition, image processing and the like), is more suitable.

Willsky's book is a very different matter. The author is a control theorist who was struck by the fact that work in digital processing and in control and estimation theory has a great deal in common, as well as certain clear differences that are themselves illuminating. There is no doubt that these two groups of subjects have much to offer one another and the author explores a range of topics from both points of view.

This kind of book is in the best sense a piece of self-indulgence on the part of the author, a leisurely examination of difficult problems and unsolved questions by a wide-ranging mind, not under any immediate compulsion to provide solutions. The result is an enjoyable and stimulating book, easy to read despite the advanced nature of the contents although

the reader is expected to be familiar with the vocabulary, verbal and mathematical, of the subject. There are five main chapters, concerned with (1) the analysis of stability, (2) parameter identification and related topics, (3) synthesis, realization and implementation, (4) multiparameter systems, distributed processes and random fields, and (5) nonlinear system analysis.

The flavour of the text is well illustrated by the opening remarks of the chapter on multiparameter systems, which is much concerned with image processing: "This area is rich in both potential applications and challenging theoretical problems . . . How does one process distributed data efficiently? What properties do recursive techniques have when the recursion is in more than one dimension? Do causality and state make any sense here? What about stability? What are the tools for analyzing stochastic processes? How do we 'predict' when time is not the independent variable? What role do recursive estimation techniques play? What are recursive estimation techniques? Which concepts concerning signals and systems in one independent variable carry over to the multiparameter case? Which do not, and why don't they?". Altogether an interesting and provocative book.

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